ABSTRACT OF THESIS

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Title of Thesis Analytical and Structural Studies on Plant Gums of the Acacia Group

Comparative Analytical Studies

Comparative analyses were carried out on a series of single nodules of the gum from Acacia seyal Del. Differences in the analyses and physical properties of both crude and purified samples were observed.

Fractionation of the gum was achieved on DEAE-cellulose, four fractions being obtained. These varied in the uronic anhydride content and rhamnose.

Structural Studies

Acacia seyal gum contains residues of D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. Graded acid hydrolysis gave a mixture of neutral and acidic oligosaccharides amongst which the following were identified:

6-O-β-D-glucuronopyranosyl-D-galactose
6-O-β-D-galactopyranosyl-D-galactose
3-O-β-D-galactopyranosyl-D-galactose
3-O-α-D-galactopyranosyl-L-arabinose
3-O-β-L-arabinopyranosyl-L-arabinose

Hydrolysis of methylated whole gum afforded

2,3,5-tri-O-methyl-L-arabinose
2,3-di-O-methyl-L-arabinose
2,5-di-O-methyl-L-arabinose
2,3,4-tri-O-methyl-L-arabinose
2,3,4,6-tetra-O-methyl-D-galactose
2,4,6-tri-O-methyl-D-galactose
2,4-di-O-methyl-D-galactose
2,3,4-tri-O-methyl-L-rhamnose
2,3,4-tri-O-methyl-D-glucuronic acid
2-mono-O-methyl-D-galactose (trace)
and 2,3,4-tri-O-methyl-D-galactose (trace)

Hydrolysis of methylated acid degraded gum afforded

2,4-di-O-methyl-D-galactose
2,3,4-tri-O-methyl-D-galactose
2,4,6-tri-O-methyl-D-galactose
2,3,4,6-tetra-O-methyl-D-galactose
2,3,4-tri-O-methyl-D-glucuronic acid

Use other side if necessary.

The periodate resistant part of the gum was also methylated and on hydrolysis the following sugars were obtained:

2,3,5-tri-O-methyl-L-arabinose
2,5-di-O-methyl-L-arabinose
2,3,4-tri-O-methyl-L-arabinose (trace)
2,3,4-tri-O-methyl-D-galactose
2,4,6-tri-O-methyl-D-galactose
2,4-di-O-methyl-D-galactose
2,3,4,6-tetra-O-methyl-D-galactose

Partial structures for the gum, in terms of the galactan framework and the acid labile periphery, are discussed in the light of these results.

The structural features of the gum have been compared with those of Acacia senegal and Acacia pycnantha.

ANALYTICAL AND STRUCTURAL STUDIES ON PLANT GUMS OF THE ACACIA GROUP

By

MAREK A. HERBICH

A THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF EDINBURGH MAY, 1963

TO MY PARENTS

CONTENTS

SECTION I (INTRODUCTORY)

(i) General Introduction

The term plant gum is frequently applied to exudations composed of terpenoid resins, carbohydrates or a mixture of both types of material which are formed after injury to the outer portions of certain trees and fruits. Their origin is obscure. There appears to be a rapid mobilization of carbohydrate materials in the cell sap near the point of injury and in a short time exudations of the gum begin in the form of droplets which gradually dehydrate giving in the first place a viscid syrup. Finally, hard glossy nodules are produced which, in many cases, are composed almost entirely of carbohydrate material with surprisingly little extraneous material. It is of significance that healthy trees tend to exude less gum than those in poor condition. For this reason it has been suggested (1) that exudation of gum is a result of infection, but there is little evidence (2) of bacterial action on the free sugars of exuded cell sap, although a few gums including chagual (3) and honey locust gum (4) are in fact known to be pathological products. It is probable, therefore, that a tree exudes gum in order to seal off the injured part and to prevent the spread of infection.

The chemistry of gum arabic has interested scientists for many years, notable early contributions being found in the work of Neubauer (5), Scheibler (6), and O'Sullivan (7). The Acacias belong to the sub-order Mimosoideae of the order Leguminosae. There are in all 500 species (8) in tropical and subtropical regions, particularly in Africa and Australia. In the Sudan there are 22 species (9). The true Acacias constitute mainly the thorn-forest in the Arid Zone, this belt of prickly shrubs and trees apparently stretching right across Africa from east to west. The life

of a tree appears to be between twenty-five and thirty years and slightly less in some places. At the later age it is attacked by numerous insects and finally destroyed by white ants and beetle borers.

Although many species of Acacia are known, commercial Acacia gums, produced largely in the Sudan and Nigeria, are produced essentially from one species, Acacia senegal. Collection of gum from only one species has led to a more uniform product and adulteration is reduced. The trees can grow on both sandy and clay soil and reach a height of 15 to 20 feet, although Acacia seyal grows to a height of approximately 30 feet.

Plant gums have long been used by man. Gum arabic was used by the Egyptians for thickening paints in the second century B.C. In modern times their use in industry has been continually increasing and to-day gums are employed for a variety of purposes. Thus they are used as adhesives, as thickeners and stabilizers for emulsions, as sizes for textiles, as slime precipitants in ore refining and as additives to numerous industrial products. The largest consumer of gums, however, is probably the foodstuffs industry and in particular the confectionery industry.

(ii) General chemistry of gums

The physical properties of gums are widely used in industry and it is surprising how few gums have been studied from a physico-chemical point of view. This may be due in part to the fact that gums are neither pure nor homogeneous. The crude gum usually contains minor amounts of proteinaceous matter of an unknown nature. Most water-soluble gums are complex polyacids which occur as the calcium, magnesium, potassium and sodium salts. It is difficult to ascertain their homogeneity, although ultra-filtration and electrophoretic studies have indicated that gum arabic (Acacia senegal), for example, is heterogeneous (9, 10). Most of the physical studies reported have been carried out on the two most important industrial gums - gum arabic and gum tragacanth.

The tears of gum arabic crumble and become opaque when dried in direct sunlight in the Sudan, but this does not destroy the gum like properties. When heated, this gum undergoes changes which are accompanied by a decrease in the viscosity of its aqueous solution. This may be due to depolymerisation, and, if the heating is sufficient, rearrangement of the glycosidic bonds by the type of transformation that has been shown to occur during the dextrinization of starches by the roasting process (11). Instability in hot aqueous solution is very noticeable with gums containing sugars such as arabinose, rhamnose and fucose (12). The viscosity of gum arabic solutions also decreases on the addition of inorganic salts (13). Another physical property attributed to gum arabic is the lowering of surface tension of water solutions (14).

A high degree of branching is a typical feature of the molecular structures of many of the gums studied to date. There is evidence indicating

the molecules to be very flexible with small attractive forces between neighbouring sugar residues (15). The general "architecture" of a gum molecule is believed to be of more importance in deciding the physical properties than the fine structure of the building units. Thus the property of "gumminess" has been attributed to an irregularity of structure which will not permit inter-molecular reactions to form three dimensional networks (gels) (16).

The molecules of most gums consist of hundreds of sugar residues incorporated in a highly branched structure, at least three different monosaccharide residues usually being present. It is not surprising, therefore, that the question of homogeneity is a difficult one. In the past, gum exudates were generally considered to consist of one polysaccharide only, because of the inability of the methods then available to effect fractionation. For a correct interpretation of the experimental facts it is important to work on homogeneous polysaccharides and the problem of achieving fractionation and detecting heterogeneity is, therefore, very important.

Heterogeneity may be of three different kinds :

(1) Each of the polysaccharides in the mixture contains different structural units.

(2) The basic structural units are the same in all molecules, but they exist in different combinations or different proportions in the various molecules.

(3) The difference is solely one of molecular size.

The latter type is probably quite common for polysaccharides, but should not prevent a correct elucidation of the structure. The first two types both cause grave difficulty, however; fractionation may be extremely

difficult. In the case of Acacia senegal, Heidelberger was able to effect fractionation by employing the delicate precipitin reaction (17). This takes advantage of the cross-reaction between certain polysaccharides and pneumococcus antisera to form a complex which precipitates. He obtained a fraction which contained a much smaller proportion of rhamnose residues than the original sample of gum. A recent method of column chromatography using diethylaminoethylcellulose as packing (18) has produced four fractions from Acacia seyal Del differing in uronic acid content as well as in amounts of certain sugars present, (see Section II, Vc).

An examination of several gum nodules from the species Combretum leonense has revealed definite internodule differences (19). Thus the uronic anhydride content of the purified polysaccharide from separate nodules was found to vary from 15% to 20%. It has been recognised for some time now that hemicelluloses may be considered as a "family" of polysaccharides containing the same basic structure and differing in their fine structure (20). It does not seem unreasonable to assume the same kind of variation in the structures of related gums or, indeed, within gums from the same species. The inter-nodule differences detected (19) in the gum of Combretum leonense may then be explained by assuming consistency in the molecular "core", with only the proportion of acidic substituents being subject to variation (21). More recent work (22) has indicated that variations only occur between nodules obtained from different trees and not between nodules from the same tree.

In view of, however, our incomplete knowledge about the homogeneity of the gum investigated so far the possibility of heterogeneity should not be forgotten when interpreting the experimental data concerning

their chemical constitution.

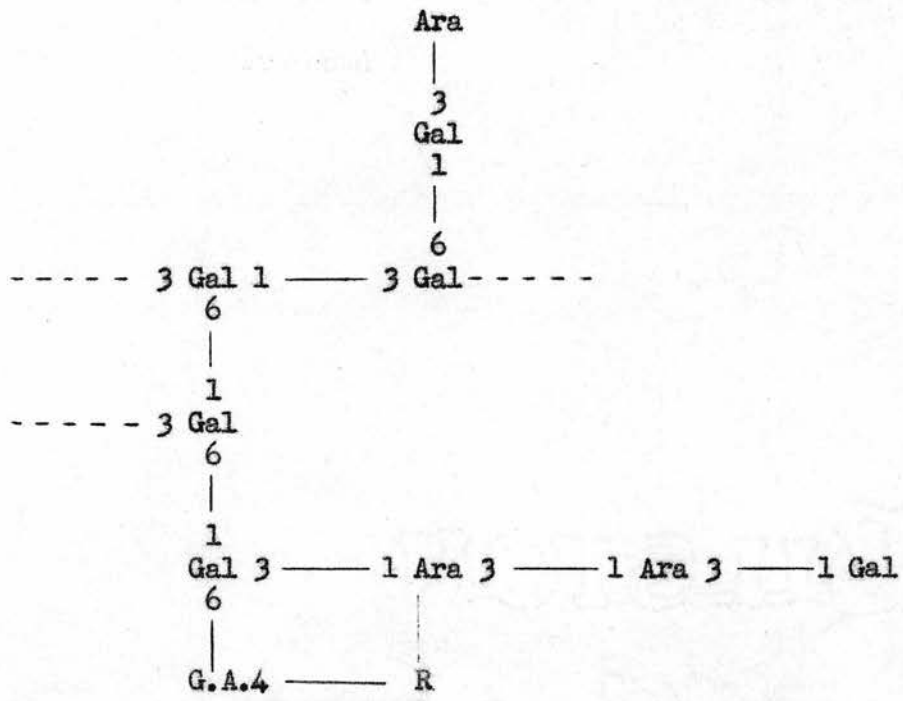
Owing to the great complexity of the chemical structures of gums, it has not yet been possible to assign a unique formula to any of those studied to date. Many structural features have, however, been elucidated, and both similarities and major differences have been discovered. A high proportion of the gums investigated have in common a relatively stable "core" of sugar residues to which are attached side-chains which may contain neutral sugars and uronic acid units. The sugar residues are joined together by glycosidic linkages, and for an understanding of the chemical structures of gums it is important to realise that uronosyl linkages are always very stable and by far the most resistant in gum molecules. Thus gums possessing the structural features mentioned may be hydrolysed to yield aldobiouronic acids together with neutral monosaccharides. Also characteristic of this large group of gums is the presence of acid-labile units such as L-rhamnose and L-arabinose, the latter generally existing in its furanose form. These acid-labile units may be removed from the polysaccharide molecules by very mild acid hydrolysis or by autohydrolysis; removal of such units gives the "degraded" gum.

The general type of structure outlined above is common to a number of gums, the most prominent of which is gum arabic. Many gums have, however, been investigated which possess structures deviating in a greater or lesser degree from the one outlined above, e.g., uronic acid residues may occur in the interior chains of the molecules. If the proportion of uronic acid residues present is very high, most of the glycosidic linkages will acquire great stability. The chemical properties,

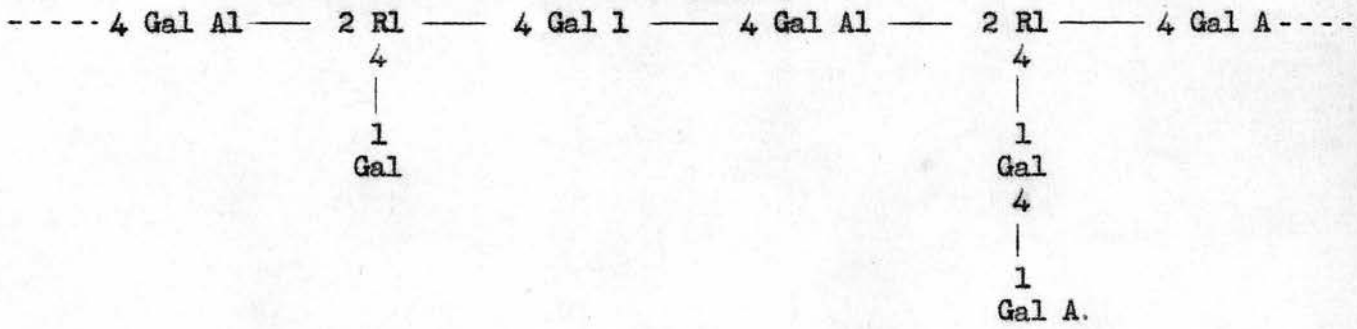
therefore, depend to a great extent on the uronic acid content of the molecules. It has also been found that D-galacturonic acid gives rise to an even more stable uronosyl linkage than D-glucuronic acid. For such gums, therefore, structural studies involving degradation to simpler fragments become extremely difficult; considerable decomposition of the liberated sugar units may occur under the drastic conditions of hydrolysis required.

Another deviation from the general type of structure represented by gum arabic is the absence of acid-labile residues as was found in Khaya grandifolia gum (23). This gum also shows other distinct differences from gum arabic. Its structure is compared below with that of gum arabic in order to illustrate the extent of the variations which may be found in the structures of gums. The structures depicted are in agreement with the experimental data, but it should be pointed out that other representations involving fine-structural differences are also possible.

Proposed Chemical Structure of Gum Arabic (24)



Proposed Structure of Khaya grandifolia Gum (23)



Explanation of symbols used in the two schemes :

Gal = D-galactopyranose

Ara = L-arabinose (mostly in furanose form)

R = L-rhamnose (pyranose form)

Gal A = D-galacturonic acid

GA = D-glucuronic acid

In such materials, differences in stability displayed by the various kinds of glycosidic bonds present, and the diversity and complexity of the structures involved, have necessitated the use of several different experimental methods. These will be discussed in the next part.

(iii) General methods employed in structural investigations of plant gum polysaccharides.

Most of the earlier structural studies on plant gums began with an examination of the modes of linkage between the monosaccharides and little or no importance was attached to the source, or authenticity nor to the heterogeneity of the gum. Recently Anderson, Hirst and King (19) showed that inter-nodule differences exist in Combretum leonense gum and this has been confirmed in the present investigation of Acacia seyal Del. Differences in the proportion of sugar residues present in the various fractions which exist in the one sample of gum can render structural studies quite meaningless. It is, therefore, considered necessary to give a general outline of analytical methods employed in the purification, fractionation and estimation of monosaccharides from the polysaccharide under examination.

The polysaccharide material in a gum usually exists as the neutral or slightly acidic salt of a complex polyacid and cations such as calcium, magnesium, sodium and potassium are mainly involved. The powdered gum is dissolved in water or if necessary in dilute sodium hydroxide, and the insoluble extraneous matter such as pieces of wood, insects and sand is filtered off. The free gum acid is then precipitated by addition of acidified organic solvents such as ethanol or acetone, leaving the low molecular weight material in solution. Further purification of the polysaccharide may be effected by reprecipitation, dialysis or by treatment with ion-exchange resins. Very often these methods fail to remove all ash content and it has been found that electrodialysis can reduce the ash content to 0.05% in cases where all the other methods

mentioned have failed. The purified polysaccharide is either freeze-dried or dried by trituration with absolute alcohol or acetone followed by drying in a vacuum pistol. This latter method has been shown (25) to be unsatisfactory owing to solvent retention by the polysaccharide. Indeed Drummond and Percival (26) found a fast moving sugar corresponding to a mono-O-ethyl sugar in the partial acid hydrolysate of the gum exudate of Albizzia zygia, which they had purified by precipitation from ethanol. This was shown to arise from solvent retention since this sugar was not detected when the gum was precipitated by propan-2-ol.

In all polymer chemistry it is most important and often a most difficult task to establish the homogeneity of the starting material. Unfortunately, complete homogeneity of a sample cannot be established directly. Several polysaccharides, including some gums, have been clearly shown to be heterogeneous. For example, gum tragacanth (27) has been fractionated into polysaccharides having entirely different chemical structures. In other investigations polysaccharides have been assumed to be homogeneous because of failure to effect fractionation by some of the available methods. This is a dangerous criterion.

Graded extraction has often been used to isolate pure polysaccharides from the raw materials. Dimethyl sulphoxide is considered (28) to be a good solvent for preferential extraction of polysaccharides especially for those containing O-acetyl groups. Another method commonly used for isolating pure polysaccharides is fractional precipitation. The two components of Olibanum gum (29) and of Khaya senegalensis gum (30) have been fractionated by this method. This type of fractionation can also be used to fractionate mixtures of acetylated and methylated (as in the case of gum tragacanth) polysaccharides. Most polysaccharides form

insoluble complexes with certain inorganic compounds and are thus precipitated from solution. Fehling's solution (31), cupric acetate (32), cupric sulphate (33), cetyl trimethyl ammonium bromide (Cetavlon) (34), and Cetavlon-boric acid mixtures (35), have all been used to effect fractionation.

Adsorption of displacement chromatography has had only little success in separating mixtures of polysaccharides. Some instances in which it has been used are the separation of mucopolysaccharides on cellulose columns by gradient elution with increasing concentrations of ethanol containing 0.3% barium acetate solution (36), the fractionation of sugar beet araban on a charcoal column (37) and of polysaccharides from Mycobacterium tuberculosis on a silica gel column (38).

A more efficient chomatographic procedure for polysaccharides has been developed recently by Neukom et al. (18). Their method, which makes use of an anion-exchange cellulose, was developed for analytical purposes, but can be extended to small scale preparative separations. They found, in analogy, to the precipitation of acidic and neutral polysaccharides with quaternary ammonium salts, that acidic polysaccharides are absorbed readily on anion-exchange cellulose at neutral pH values, whereas neutral polysaccharides are not absorbed or only weakly absorbed. Thus by using diethylaminoethyl-cellulose in different forms (borate, phosphate, hydroxyl etc.) together with a suitable elution medium at different pH values or different molar concentrations, they were able (18) to fractionate materials such as wheat flour polysaccharides. They have also shown that, for pectic substances, the extent of absorption on the cellulose derivative depends on the degree of esterification, the degree of polymerisation and the content of side groups (39). The

ultrafiltration technique, using membranes of graded pore size, has been used by Jones (40) to separate dextran into fractions of different molecular weights. A more recent method, developed by Pharmacia in collaboration with the Institute of Biochemistry of the University of Uppsala, makes use of a substance (Sephadex) made by crosslinking the polysaccharide dextran. It consists of a three-dimensional network of polysaccharide chains and is made with different degrees of cross-linkage which determine the porosity of the network. This gel filtration technique has been applied in studies on proteins, enzymes, and polysaccharides (41, 42, 43, 44, 45).

There is no unambiguous method to assess the homogeneity of polysaccharide obtained by the above methods of fractionation. Chemical analysis (quantitative and qualitative) of component sugars and uronic acids and determinations of physical constants of the polysaccharide (e.g. optical rotation, viscosity, molecular weight distribution) are often useful to detect differences between fractions.

Electrophoresis (ionophoresis) using glass fibre paper (this has advantage over filter paper in that no adsorption of polysaccharide takes place) and borate buffer or 2N-sodium hydroxide has been used in a few instances to test the homogeneity of polysaccharides. Using this technique, gum ghatti, gum tragacanth, the gums of Acacia pycnantha, Acacia senegal and Acacia arabicum have been claimed to be heterogeneous since they all showed more than one spot (9).

Another important analytical test for homogeneity depends on the fact that antipneumococcus horse sera will give precipitates with polysaccharides which are structurally related to the antipneumococcus polysaccharides. Thus gum arabic gives a precipitate with Type II antipneumococcus serum from which a polysaccharide having less rhamnose than the original

gum can be recovered (17).

The molecular weight distribution of a mixture of polysaccharides can be investigated by sedimentation analysis in an ultracentrifuge (46). This method also gives an estimate of the molecular weights of the components. Molecular weight distribution can also be studied by turbidimetric measurements using light-scattering photometers.

In view of the possibility of heterogeneity in gums, caution must be exercised when interpreting experimental results. Thus most, if not all, interpretations, especially those regarding the fine structure of polysaccharides, can indicate only the most probable structure; absolute or unique assignments may never be possible.

Having thus established the general nature of the polysaccharide by determination of (a) its physical constants (such as optical rotation, neutralisation equivalents and viscosity coefficients) and (b) its chemical composition (e.g. its hexose, pentose, uronic anhydride, acetyl, methoxyl, nitrogen and ash contents) detailed structural studies may begin. These involved, in the main, the nature of linkages of the monosaccharides in "core" of the gum and in its side-chains. The three most successful methods developed in this connection are methylation studies, partial acid hydrolysis and periodate oxidation followed by reduction and methylation of the resulting products.

Methylation Studies

This classical method of investigation in the carbohydrate field makes use of the fact that hydroxyl groups in sugar residues may be etherified. The ether group generally introduced is the methoxyl group. Because of its relatively small bulk the effects of steric hindrance are minimised as much as possible.

The principle underlying this method depends on the complete methylation of all the hydroxyl groups which are not involved in any glycosidic linkages. Hence on hydrolysis of the resulting methylated polysaccharide, the methyl sugars obtained will carry free hydroxyl groups only on those carbon atoms which were involved in glycosidic linkages or ring formation. Identification of these partly methylated sugars will then provide evidence of the nature of the sugar residues, and of their modes of linkage in the polysaccharide if the ring size of the sugar residues is known. The methylation results will also indicate the non-reducing terminal residues, the points at which branching occurs, and the number of residues per average building unit. Thus for homopolysaccharidesit is possible to construct the building unit, with fair significance, from methylation results alone. In the case of heteropolysaccharides, however, it is necessary to know the order in which the different sugar residues occur before such a unit can be constructed.

The standard method of methylation is still the one developed by Haworth (47) using dimethyl sulphate and 30% NaOH. This method rarely gives full methylation, especially with acidic polysaccharides. Methylation can be completed by Purdie's method (48) using methyl iodide and silver oxide or by Kuhn's method (49) which uses barium oxide and methyl iodide in N,N- dimethyl formamide as solvent.

Other less commonly used methods available for methylation make use of thallium hydroxide and methyl iodide (50), diazomethane (51), and methyl iodide with sodium in liquid ammonia (52). The last method has been adopted to the micro-scale by Isbell et al. (53).

The fully methylated polysaccharides are insoluble in hot inorganic acids and therefore cannot be directly hydrolysed. This

difficulty may be overcome by preliminary methanolysis or formolysis followed by hydrolysis. The products of hydrolysis can be separated by various chromatographic techniques and the individual methylated sugars characterised by means of crystalline derivatives. Very recently Aspinall (54, 55) has used the method of gas chromatography for identifying individual methyl glycosides in the presence of others.

Partial Acid Hydrolysis

Acid hydrolysis under various conditions is used to isolate simple fragments of the molecule whose structures may be completely determined, thus providing information about the order and mode of linkages of the monosaccharides in the polymer. In addition to the sequence of sugar residues, the sequence of glycosidic chain linkages and the anomeric configuration of the linkages in the polysaccharide can be determined by this method. The glycosidic linkages in a polysaccharide display wide differences in stability, enabling one to carry out a stepwise degradation of the polysaccharide. It is, therefore, possible to get information on different portions of the molecule such as the side-chains and the acid resistant "core".

Sugars existing in the furanose ring form are much more acid-labile than those in the pyranose form. Thus furanose sugars are easily removed by heating a solution of the polysaccharide with very dilute acid (ca. 0.01N) or, in the case of an acidic polysaccharide by heating an aqueous solution. This latter process is known as autohydrolysis. Methylation of the degraded polysaccharide remaining after removal of the acid-labile groups will yield information regarding the structure of the "core" of the polysaccharide.

The structural significance of oligosaccharides present in minute

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The structural significance of oligosaccharides present in minute

quantities in the partial acid hydrolysates are doubtful, because acid hydrolysis is a reversible reaction. Thus when an acidic solution of one or more monosaccharides is heated, oligosaccharides are formed; these are termed "reversion products". For example, Jones and Nicholson (56) have found that L-arabinose in hydrochloric acid gives three reversion products and the presence of these in hydrolysates which contain arabinose indicates that reversion may be taking place. Certain samples of gum arabic on mild hydrolysis yield 3-O-β-L-arabopyranosyl-L-arabinose. The absence of the other two reversion products, however, is evidence in favour of it being a true hydrolysis product. Reversion products can generally be distinguished since their concentration reaches an equilibrium value, whereas actual fragments of the polysaccharide are hydrolysed on more prolonged heating.

The differing stability of the glycosidic linkages in polysaccharides, which allows a great degree of selectivity on hydrolysis, also has disadvantages. Thus it is difficult to determine the nature of the most acid-labile linkages since these will be the first to be cleaved on acid hydrolysis and will therefore not be present in any of the oligosaccharides. In such cases resort is made to acetolysis. Thus the acid-labile rhamnose residue in *Acacia senegal* has been shown to be attached to position 4 of the glucuronic acid by isolation of the oligosaccharide 4-O-L-rhamnopyranosyl-D-glucose from partial acetolysis products of the reduced gum (57).

A novel method of determining the mode of attachment of very acid-labile groups to the adjacent sugar residues has been developed by Aspinall *et al.* (58). This is done by conversion of acid-labile furanoside linkage in polysaccharide to the acid resisting furanosiduronic acid linkage by oxidation with oxygen in the presence of a

platinum catalyst. It is then possible to isolate and identify aldobiouronic acids from the acid hydrolysate of the oxidised polysaccharide, indicating the nature of the original glycosidic linkage. In contrast, it is not possible to hydrolyse polyuronides such as pectic acid, alginic acid or gums of high uronic acid content without considerable degradation. These polysaccharides are more easily hydrolysed if they are converted to the corresponding neutral polysaccharide by reduction.

In comparison to acid hydrolysis, enzymic hydrolysis (enzymolysis) has the advantage of specificity. For example the action of β-amylase on 1-4'-linked unbranched glucan amylose gives maltose as the only final product. On the other hand, acid hydrolysis of amylose gives glucose and maltosaccharides arising through random scission of bonds. The use of enzymes on heteropolysaccharides containing a diversity of building units and linkages has been restricted. Even so, since a pure enzyme will split only one particular bond out of a variety, it would be possible to obtain fragments of great structural significance.

The mixture of neutral oligosaccharides can be separated from monosaccharides and from one another by chromatography on charcoal-celite (59), cellulose (60) or resin columns[c] (61). The acidic oligosaccharides are separated from the neutral sugars by absorbing[b] them on weakly basic anion exchange columns, washing the column with water until free of neutral sugars. The absorbed acidic oligosaccharides may then be fractionated by eluting the column with increasing concentrations of formic acid. A further separation of the oligosaccharides may be effected on thick chromatography paper.

The component sugars of the oligosaccharides are identified by hydrolysis, or by reduction followed by hydrolysis if it is an acidic

oligosaccharide. It is also possible to detect the reducing end group of the oligosaccharide by first reducing it to the glycitol followed by hydrolysis and identification of the products. An idea as to the identity of the oligosaccharide can be obtained from its chromatographic speed in different solvent systems, ionophoretic behaviour and optical rotation. The nature of the linkages can be established by methylation and if the quantities are very small the products of methanolysis can be identified by gas-liquid partition chromatography. Periodate (62) and lead tetra-acetate (63) oxidations can give information as to type of linkage depending on the type of substitution of reducing residues.

Quantitative aspects of the products of partial hydrolysis are also liable to be misleading on occasion. Thus the proportion of an oligosaccharide released depends on the stability of its internal glycosidic linkage (s) and on the firmness with which it is incorporated in the molecule in addition to its quantitative significance as a structural feature in the polysaccharide.

Periodate Oxidation

The ability of the periodate ion to cleave the carbon-carbon bond in αβ-glycols (64) is used in structural studies on both simple and complex polysaccharides. The reaction seems to occur via an intermediate complex in which the two hydroxyl groups are held in cis-position (62); in compounds where the adjacent hydroxyl groups are rigidly held in a trans-position, no cleavage takes place (65).

The reaction is generally carried out in aqueous solution at room temperature in the absence of light. It is now widely used and it is becoming more and more obvious that the oxidation is not as simple as had been assumed. The reaction seems to be influenced by temperature, pH,

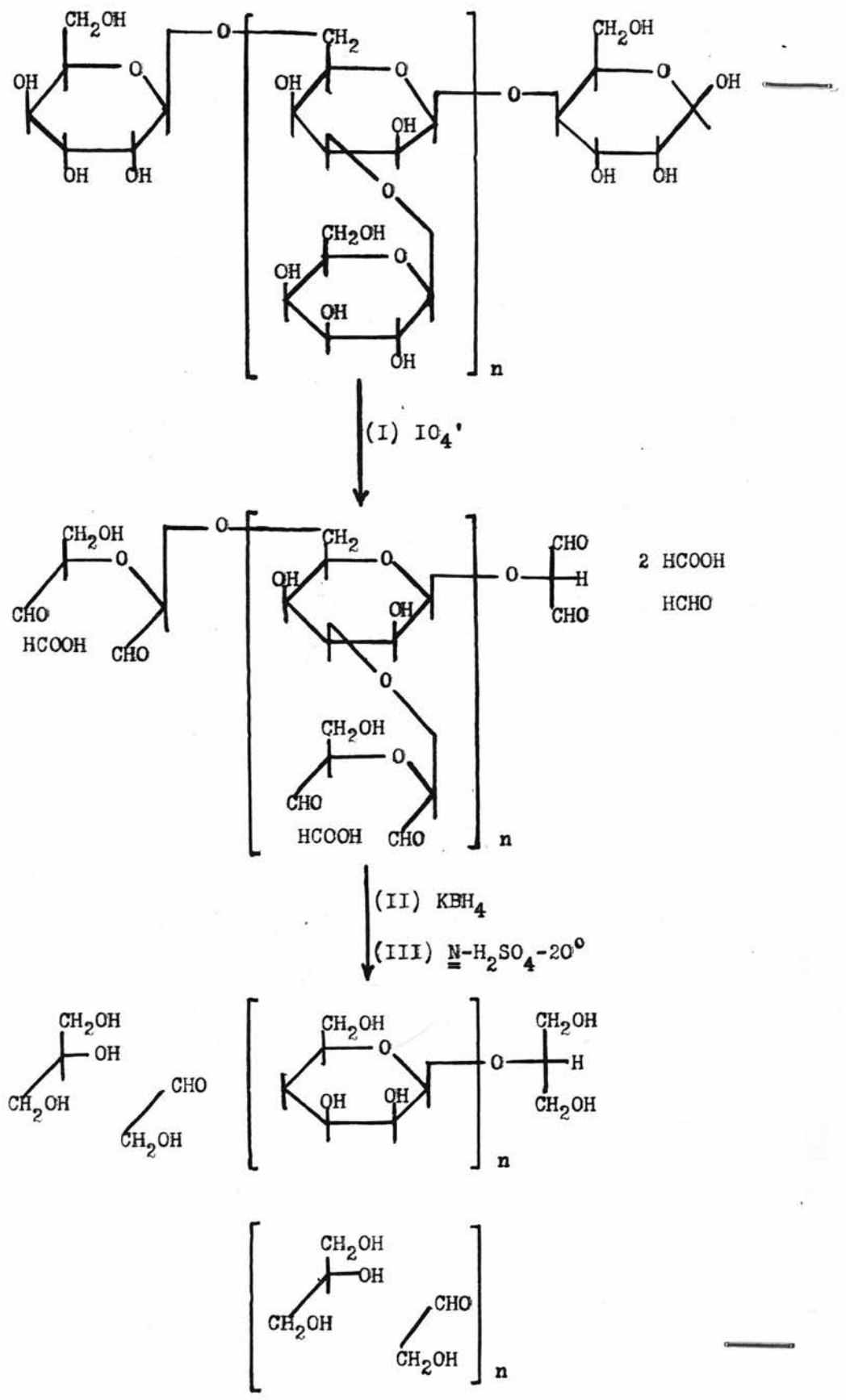
concentration of reactants and light (62). The essential features of the periodate oxidation are shown in the scheme below.

It should be pointed out that the di-aldehyde formed on cleavage of the carbon-carbon bond does not seem to be as depicted but forms a ring closure (66), although in certain reactions they do behave as aldehydes. Also, the formation of formaldehyde as shown depends on the formyl ester linkage at C1 being broken by hydrolysis. Since formaldehyde may only arise from the terminal residue at the reducing end of the polysaccharide, this fact has been used to determine molecular weights.

When three carbon atoms are linked together and all carry a free hydroxyl group, the two C-C bonds are broken with the formation of one molecule of formic acid. Estimation of formic acid will therefore afford a measure of the frequency with which such a structural feature occurs. Only terminal sugar residues and 1,6-linked hexopyranose residues may possess three adjacent hydroxyl groups and it may therefore be possible to assess the degree of branching in certain polysaccharides.

Since one molecule of oxidant is consumed for each αβ-glycol group cleaved, it is possible, therefore, by estimating the amount of periodate reduced, to distinguish between certain alternative structures. If a linear polysaccharide is known to consist of hexopyranose residues linked through their 1,4- and 1,6-position only, the proportion of each may be calculated from the periodate uptake because they consume one and two molecules of oxidant respectively (67). The periodate is usually estimated by a standard arsenite solution (68) or more recently by a spectrophotometric method (69).

In the application of periodate oxidation to polysaccharides, advantage may frequently be taken of the fact that some of the sugar units



General Scheme Showing Periodate Oxidation Followed by a Smith "Degradation" on a Polysaccharide.

are not susceptible to attack by the reagent since they do not possess adjacent hydroxyl groups. Thus for heteropolysaccharides such as gums the proportion of unoxidisable residues of each type of monosaccharide may be determined. The sugars are last estimated after hydrolysis of the derived polyalcohol, since the polyaldehydes on hydrolysis give rise to interfering products. A valuable extension of this method was recently developed by F. Smith (70) and involves the conversion of the polyaldehyde to the corresponding polyalcohol by borohydride reduction with subsequent treatment with dilute mineral acid at room temperature. Only the hemi-acetal linkages may be broken during this mild hydrolysis. If any polysaccharide remains after this degradation it is separated from the rest of products by precipitation with an organic solvent and then subjected to methylation or partial hydrolysis studies.

In view of the complexity of the molecular structures of gums, methods which selectively degrade gums to simpler polymers - such as the Smith (70) and Barry (71) Degradations - are particularly valuable.

(iv) Molecular structure of Acacia Gums

To date, the gums from seven species of the genus Acacia (family Leguminoseae) have been studied; each has been found to contain the same sugar residues, namely, D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid in different proportions. The gums also undergo autohydrolysis with liberation of L-arabinose and a disaccharide. Some rhamnose may also be liberated, but it is generally less readily removed than arabinose. The degraded gums consist of D-galactose and D-glucuronic acid residues but the uronic acid units are not necessarily confined to the resistant part of the polymer. They may also form an integral part of the side-chain, since in Acacia karroo (72) and Acacia cyanophylla (73) gums, an aldobiouronic acid is liberated during autohydrolysis; in the case of the latter gum, the aldobiouronic acid so produced is the same as that obtained by hydrolysis of the degraded gum.

Recently, also Aspinall (57) has reported the isolation of 4-O-L-rhamnopyranosyl-D-glucose from Acacia senegal after a series of reactions involving acetylation and reduction, thus giving support to the theory of the existence of D-glucuronic acid residues in the periphery of the structure.

The same aldobiouronic acid, 6-O-β-D-glucuronosyl-D-galactose has been detected in all members so far examined, whilst in Acacia karroo gum a second aldobiouronic acid, 4-O-$\overset{\alpha}{\beta}$-D-glucuronosyl-D-galactose has been found. Acacia karroo gum may either be heterogeneous, or it may differ from the other species in containing two aldobiouronic acid residues.

Of this genus, only Acacia senegal and Acacia pycnantha have been fully methylated and extensively investigated prior to the present work. In all cases a branched structure with 1,3- and 1,6-linked galactose is indicated. The results of total and partial acid hydrolysis and methylation studies are summarised in the Tables I, II and III.

TABLE I

Molar Proportions of Sugar Residues in Acacia Gums

Species	Gal	Ara	Rha	G.A.	Ref.
Senegal	3	3	1	1	74,78
Cyanophylla	11	2	5	5	73
Mollissima	5	6	1	1	75
Catechu	9	4	3	3	76
Pycnantha	40	20	1	6	77
Karroo	28	24	1	6	72
Seyal	10	12	1	3	p.38
Sundra	41	27	14	18	79

KEY: Gal = D-galactose

Ara = L-arabinose

Rha = L-rhamnose

G.A.= D-glucuronic acid

p = pyranose

f = furanose

TABLE II

Disaccharides Isolated from Acacia Gums

Species	Disaccharides isolated after autohydrolysis	Disaccharides isolated after acid hydrolysis	Aldobiuronic acid isolated after acid hydrolysis	References
Senegal	Gal 1—β—3 Ara	Gal 1—β—3 Gal Arap 1—β—3 Ara	G.A.1—β—6 Gal	74,80
Cyanophylla	Gal 1—α—3 Ara		G.A.1—β—6 Gal	73
Mollissima	Arap 1—β—3 Ara		G.A.1—β—6 Gal	75
Karroo	Ara 1—β—3 Ara		G.A.1—β—6 Gal G.A.1—α—4 Gal	72
Pycnantha		Gal 1—β—3 Gal Araf 1———3 Ara	G.A.1—β—6 Gal	77,81
Seyal	Arap 1—β—3 Ara	Gal 1—β—3 Gal Gal 1—β—6 Gal Gal 1—β—3 Ara	G.A.1—β—6 Gal	
Sundra			G.A.1—β—6 Gal	79

TABLE III

Products Isolated after Hydrolysis of Methylated Acacia Gums

Species	G.A.	Gal.	Ara.	Rha.	Ref.
Senegal	2,3-	2,4-	2,5-	2,3,4-	74
	2,3,4-	2,3,4,6-	2,3,5-		
			2,3,4-		
Seyal	2,3,4-	2-	2,3-	2,3,4-	Sect III (iii)
		2,4-	2,5-		
		2,4,6-	2,3,5-		
		2,3,4-(trace)	2,3,4-		
		2,3,4,6-			
Pycnantha	hexamethyl ether of G.A.1$\overset{\beta}{\text{---}}$6 Gal	2-, 2,3-	2,3-(trace)	2,3,4-	82
		2,5-, 2,4-	2,5-		
		2,4,6-	3,5 (trace)		
		2,3,4-	2,3,5-		
		2,3,4,6-			

2,6-

Object of Present Investigation

Acacia seyal Del is of considerable interest, being second only to *Acacia senegal* (syn. *verek*) in commercial importance. In this work it was intended to carry out a detailed analytical and structural investigation on *Acacia seyal*, and to compare its structural features with those of the other *Acacia* species, which have been studied to date.

Such comparisons will enable one to understand the structural relationships of very complex gums belonging to related botanical species.

EXPERIMENTAL

General Experimental Methods

Moisture contents were found by heating to constant weight at 103°.

Ash content (%) was found by heating in a muffle at 550°; constant weight was reached in 4 hours.

Nitrogen (%) was found by a semi-micro Kjeldahl method.

Anderson's apparatus (83) was used to determine uronic acid content.

Free titrable acidity was found by direct titration with 0.1N NaOH to phenolphthalein end-point and also by measuring the pH and plotting a graph of pH/vol. of 0.1N NaOH added.

Viscosity determinations were carried out in a modified Ubbelohde viscometer which had a flow time for water of 218 sec. at 25°C. All measurements were carried out in a thermostatically controlled water-bath (constancy = $\pm$ 0.1°C). Flow times were measured by a stop-watch and the results expressed using gm/ml as concentration units. No kinetic energy corrections were applied as the instrument used was designed to minimise the error involved. All measurements were carried out on aqueous solution of the gum containing 4% NaCl; subsequent dilution was with 4% aq. NaCl solution.

Methoxyl determinations (84) were carried out by the vapour-phase infra-red method which distinguishes yields of methyl iodide from other volatile products arising from solvent retention, decomposition etc.

Optical rotations of substances were measured in aqueous solution at 20° $\pm$ 1°.

Paper Chromatography - was carried out by the descending technique using the following solvent systems (v/v)

A Benzene, butan-1-ol, pyridine and water (1,5,3,3, upper layer)

B Ethyl acetate, pyridine and water (10, 4, 3)

C Ethyl acetate, acetic acid, formic acid and water (18, 3, 1, 4)

D Butan-1-ol, ethanol and water (4, 1, 5, upper layer).

E Butan-1-ol, ethanol and water (3, 1, 1).

F Benzene, ethanol and water (11, 3, 1, upper layer).

G Butan-2-one, half saturated with water.

H Butan-2-one, acetic acid and water (9, 1, 1).

Sugars were located by heating after spraying with either saturated aqueous aniline oxalate solution or with a solution of 3% p-anisidine hydrochloride in 80% butanol / 20% ethanol. All sugars were identified by comparison with reference sugars run on the same chromatograms. The symbols used in connection with paper chromatography are

$$R_{Gal} = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by galactose}}$$

$$R_{G} = \frac{\text{Distance travelled by substance}}{\text{Distance travelled by 2,3,4,6-tetra-O-methyl-D-glucose}}$$

Separation of a mixture of sugars was carried out on thick chromatographic paper (Whatman 3 mm.); sugars were eluted by cold water; methylated sugars were eluted with methanol.

Column Chromatography.

Cellulose columns were prepared from a slurry of cellulose in acetone. The column was then washed with ethanol, water and finally with the solvent to be used.

The solvents were carefully purified as below: -

(a) Butan-1-ol was refluxed for 2 hours with potassium hydroxide (1% w/v) and then distilled.

(b) Light petroleum (b.p. 100°-120°) was shaken several times with conc. sulphuric acid (10% v/v), washed free of acid and distilled.

(c) Butan-2-one was distilled.

Preparation of charcoal/celite columns was carried out as follows:

Activated charcoal was washed by decantation with hot water. Celite (grade 545) was washed with hot concentrated hydrochloric acid : water (1:1) and then successively with water, 3% aqueous sodium bicarbonate, and water. Equal weights of charcoal and celite were mixed well in a slurry which was then poured into a glass column. The slurry was added in several portions each being allowed to settle before addition of the next. Before applying the sugar mixture, the column was thoroughly washed with water.

Gas-liquid partition chromatography.

This was carried out on a "Pye Argon Chromatograph", using argon as the mobile gas phase. The stationary liquid phase was supported on Celite and consisted of:

(a) Apiezon M.

(b) Butanediolsuccinate polyester.

(c) Polyphenol.

Operating temperatures used were 150° for (a), 175° for (b) and 200°C for (c). The T (retention times) values of the methyl ether methyl glycosides are relative to that of methyl-2,3,4,6-tetra-O-methyl-β-D-glucopyranoside.

Periodiate-Benzidine Spray (85)

Glycitols were detected by spraying the chromatogram with an aqueous solution of sodium periodate (0.2%), air drying for 5 minutes, then dipping in an ethanolic solution of benzidine (0.25 g in ethanol (80 ml) and acetic acid (20 ml)). Any sugar which reacts with periodate shows up as a white spot on a blue background, which fades after a few days.

Methylations by Kuhn's method (86) were used for very small amounts of sugars (5-50 mg) and as secondary methylations after treatment with dimethyl sulphate and sodium hydroxide. The carbohydrate material was dissolved in dimethylformamide to which was added methyl iodide. The mixture was stirred vigorously (magnetic stirrer) and barium oxide was then added together with a very small amount of barium hydroxide. The stirring was continued for 2-12 hours (depending on the amount of carbohydrate material). The contents were filtered, the residue washed with dimethylformamide and chloroform, and the combined filtrates shaken with water. The water layer was further washed with chloroform (3-4 times), and the latter, combined with the original layer, was then dried over sodium sulphate (anhydrous), evaporated to small volume and poured into petroleum ether to precipitate the anhydrous methylated carbohydrate material.

Methanolysis was carried out by heating the sugars with dry methanolic hydrogen chloride (3%) in a sealed tube for 6-12 hours on a boiling water bath. The solution was neutralised with silver carbonate and filtered; the silver carbonate and silver chloride were washed with acetone, and the combined filtrates taken to dryness under vacuum.

Demethylations (87) were carried out by dissolving the methylated sugar (5 mg) in dry dichloromethane (2 ml) cooled in an acetone / CO_2 mixture. Boron trichloride (2 ml), previously cooled to -80°, was then added. The mixture was then sealed in a glass tube and kept at -80° for 30 minutes before being allowed to come to room temperature. After a further 16 hours, the tube was opened and the remaining liquid mixture was removed under reduced pressure. The remaining solid was extracted with methanol (3 x 3 ml) then examined by paper chromatography.

Reduction with potassium borohydride.

The substance (2 mg) was dissolved in water (0.5 ml) and potassium borohydride (10 mg) in water (0.5 ml) added. The reaction mixture was left at room temperature overnight. De-ionisation was effected by treatment with Amberlite resin IR-120(H), followed by several evaporations with methanol.

Aniline derivatives of sugars were prepared by refluxing the sugars with equimolecular amounts of freshly distilled aniline in dry ethanol for 20 minutes with the exclusion of light. The syrups obtained on the removal of the solvent were allowed to crystallise and the products were recrystallised from the stated solvents.

In the preparation of aldonolactones, the sugar was oxidised with excess bromine for 48 hours. Excess bromine was then removed by aeration, the solution neutralised with silver carbonate, treated with hydrogen sulphide and evaporated to dryness. The organic material was extracted with hot acetone : water and recrystallised from ethanol.

Aldonamides were prepared from the lactones which were then dissolved in dry methanolic ammonia (prepared by bubbling ammonia gas into dry ice-cooled methanol until saturation) and left in the ice-box for 2 days. Evaporation of the solvent gave the crystalline amide which was recrystallised from stated solvent.

Phenylosazones. The sugar (ca. 10 mg) was heated for 30 minutes on a boiling water-bath with 0.01 ml of each of phenylhydrazine and glacial acetic acid, water (0.25 ml) and a drop of saturated sodium bisulphite solution. On cooling and adding water (ca. 1.5 ml) the phenylosazone was precipitated and was recrystallised from boiling water.

Phenol-sulphuric acid method for estimation of sugars (88).

The reagent was made by dissolving AR. phenol (80 g) in 20 ml of water (distilled and copper free). Various volumes (0.05-1.00 ml) of the polysaccharide solution were pipetted into test-tubes and diluted to a known volume of water. Phenol solution (0.1 ml) was added to each, followed by conc. H_2SO_4 (5 ml AR.), the stream of the acid being directed on to the solution surface so as to let the temperature rise to the maximum and to facilitate mixing. The tubes were allowed to stand for 10 min, shaken and placed for 10-20 minutes in a water-bath at 25°-30°C. The optical intensity was now measured with a Unicam S.P. 500 at 485 mμ against a blank prepared with water (1 ml). The standard curve was obtained by plotting a known weight of polysaccharide (in mg/ml) against the optical intensity.

Most of the uronic anhydride determinations were carried out by decaroxylation with 19% HCl (83), but towards the end of these studies determinations were made by decarboxylation in 55% hydriodic acid (sp. gr. 1.70) with Infra-red determination of carbon dioxide evolved (89).

SECTION II

COMPARATIVE ANALYTICAL STUDIES ON ACACIA SEYAL NODULES

As an essential preliminary to studies of the chemical structure of Acacia seyal gum individual nodules of authenticated origin have been examined as crude gum and after purification by (i) precipitation, (ii) electrodialysis and (iii) ion-exchange. As a result of specific immunological (90) reactions and electrophoresis studies (9, 10), it is now accepted (8, 16) that gum arabic (A. senegal syn verek) is a mixture of polysaccharides of similar composition; no single over-all formula has any significance, and only general features can be indicated. Early studies have been criticised (8, 16, 91) on the grounds that composite commercial samples, inadequately authenticated, were used. Although it had been suspected, despite some evidence to the contrary (92), that different samples of certain plant gums varied in chemical constitution, the possible range of variation was not known until single nodules of C. leonense were studies (19). The results implied that fine-structural differences exist from nodule to nodule, so that alcoholic precipitation of bulk material from an aqueous solution of many nodules produces a complex mixture of closely similar polymeric systems. Whenever sample size permits, it is therefore clearly desirable to assess the extent of the inter-nodule variation and to make structural studies on the simplest form of the polymer available i.e. that given by a single nodule which itself may be polymolecular and/or polydisperse (terminology as in ref. 93).

Before studying the chemical structure of A. seyal gum, a number of authenticated nodules have been investigated to find if any and if so to what extent these nodules vary in properties, composition and in heterogeneity.

Collection and origin of specimens. Mr. P. Vidal-Hall, Gum Research Officer to the Sudan Government, collected suitable gum nodules from the red-barked A. seyal Del. (a close variant, A. seyal var. fistula, has a grey bark), and kindly made them available for research. A. seyal is not normally "tapped", and the nodules originate from "natural exudation". The nodules, taken only from trees which could be authenticated, were packed individually and despatched in sealed tins. Nodules I-VI were collected at Umm Ruaba Forest Reserve, Eastern Kordofan, on 9th March, 1960; nodules VII and VIII from El Ain Forest Reserve, Central Kordofan, on 9th January, 1961. The samples studied were therefore collected from widely different areas in different seasons. Sample IX was a representative bulk sample of first quality commercial "gum talh" (A. seyal). Nodules I-VIII ranged in weight from 40-80 g; their colour varied from pale yellow to dark brown. Nodules I-IV, VII and VIII were clear and glassy, of spherical shape. Nodules V and VI were elongated and had a characteristic glazed appearance, which, we have since observed, results when nodules plasticise slightly on heating at 90-100°. It therefore appears that Nodules V and VI had been subjected to more vigorous natural drying conditions than the other nodules; it is unlikely that they were products of an earlier season, since A. seyal nodules (unlike A. senegal) become brittle through dehydration and fall from the branches within a few months (97).

Analytical Methods. The standard methods have been described. Paper partition chromatography; determination of sugars liberated on hydrolysis; autohydrolysis; electrophoresis; and viscosity experiments were carried out using the apparatus and procedures described.

Studies on crude material. The nodules, individually crushed to pass a 30-mesh sieve, gave the results shown in Table IV.

Autohydrolysis at 85-90° of 1% solutions of nodules III, V and VIII gave arabinose, together with traces of galactose and an oligosaccharide. As was observed for C. leonense gum (19), the increase in reducing power (see Fig. I) varies from nodule to nodule. The acidity of the autohydrolysis solutions (pH 4.6) did not increase appreciably with time of heating (cf. C. leonense (19), which had an appreciable acetyl content) and extensive decomposition of the liberated sugars did not occur. The viscosity of the solutions fell rapidly during autohydrolysis.

Purification of crude gum. A portion of each crushed nodule was shaken with cold distilled water to give a 2% solution which was filtered through acid-hardened filter-paper. The solutions were acidified (0.1N in hydrochloric acid); addition of acetone (4 volumes) gave a white curdy precipitate which was removed by centrifugation. Further precipitation did not occur when the clear supernatant was poured into acetone. This purification process was repeated a further 3 times: the purified gum was then dialysed against distilled water and freeze-dried.

Studies on samples purified by precipitation. The results obtained are compared in Table IV. For the determinations of the limiting flow-time number, 2% saline was found to give adequate suppression of the electroviscous effect. Although the uronic anhydride content of each sample was greater than that in the crude gum, indicating the elimination of some 5-10% of non-uronide contaminants, the precipitation processes had not reduced the nitrogen content, and the ash contents were not significantly reduced. Other purification methods were therefore investigated. It is

well-known that di- and tri-valent ions can cause gel-formation and cross-linking effects (8); ash-free samples are therefore required for some analyses (cf. ref. 93).

Purification by electrodialysis. A portion of each crushed nodule was electrodialysed (94) (as 2% aqueous solution) using ion-exchange membranes (95). Cooling coils in the electrodialysis compartments prevented the temperature of the gum from rising above 30°. Trial experiments (on sample IX) showed that electrodialysis for 6 hr. (cf. ref. 7) was required to achieve the low ash values shown in Table MI. Since ash determinations at the 0.01% level consume relatively large amounts of material, determinations were not made on all samples.

After electrodialysis for several hours, the gum solutions separated into a clear, colourless upper layer and a viscous, slightly coloured lower layer. The upper layer was removed by pipette and found to contain only trace amounts of gum. This effect may be worthy of further examination, since disintegration of a complex coacervate may be involved (cf. ref. 8).

Studies on electrodialysed samples. The results obtained are compared in Table MI. Although the ash content had been effectively reduced, no elimination of nitrogen was achieved. Potentiometric titrations showed that the ash-free gum behaved as a strong acid (pH of a 1% of solution = 2.9) and the values obtained for the neutralisation equivalent indicated that all the acidity arose from the uronic acid groups (e.g. for sample VIII: Found neut. equivalent = 1340, % uronic anhydride = 13.5): reqd. NE.= 1300 if all acidity due to uronic acid groups.

Purification by ion-exchange. A dilute aqueous solution of nodule III was filtered, then de-ionised (3) by passage through a column of the cation

exchange resin "Zeokarb 225". The freeze-dried eluate gave: ash = 2.4%, nitrogen = 0.14%, uronic anhydride = 13.5%, $\alpha_D = +59^\circ$. Viscosity determinations gave the plot shown in Fig. V. This ion-exchange method was not applied to the other samples since the purification achieved did not approach that given by electrodialysis.

Comparison of the viscosity behaviour of samples before and after purification. Samples were examined carefully to assess (a) the extent of inter-nodule variation, (b) the effect on each nodule of the various purification procedures. The viscosity plots for the crude and purified samples of nodules I to VIII are shown in Figs. II, III and IV.

Hydrolysis : percentages of sugars present.

Total hydrolysis with 2N H_2SO_4 at 90° gave D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid plus a faint pink spot travelling in the region of 2-O-methyl arabinose which could not be identified. A known weight of ribose (100 mg) was added as a reference sugar to a sample of the gum (900 mg) was which then subjected to hydrolysis with 2N H_2SO_4 for 8 hours at 90°. The solution was partly reduced in acidity by $Ba(OH)_2$ and finally neutralised with $BaCO_3$, de-ionised (Zeo-Carb 225 resin) and reduced in volume to a syrup. After chromatographic separation on Whatman paper (3 MM), the sugars were eluted and estimated by Somogyi's method (102). Expressed as percentages, sample IX gave galactose 38%, arabinose 46%, rhamnose 3% and glucuronic acid 12.5%. It is considered that the results cannot be more accurate than within $\pm$ 5 to 10% of the actual percentage present.

For samples I - VIII, however, the results, particularly for the rhamnose content, varied by amounts which were considered to be outside the possible experimental error. The two most widely differing nodules were samples V and VII. Sample V gave 11% glucuronic acid, 42% galactose,

47% arabinose and 1% rhamnose. Sample VII gave 16% glucuronic acid, 34% galactose, 42% arabinose and 8% rhamnose.

Equivalent weight determination

This was carried out by two methods: (a) by direct titration with 0.1N NaOH to a phenolphthalein end point and (b) by titration with 0.1N NaOH against change of pH. This method was rather longer but results obtained coincided with those obtained by procedure (a) as summarised in Table VII.

Fractionation experiments on aqueous solutions of the gum.

1. Chemical precipitation methods. No useful fractionation resulted from (a) the graded addition of ethanol, (b) addition of iodine-potassium iodide reagent (cf. ref. (88)), (c) addition of cetyltrimethylammonium bromide (34) at pH 4, 7 or 9.

2. Electrophoresis. Several experiments were made, using glass-fibre paper in 2M sodium hydroxide at 1,000 V for 6-18 hr. Movements of several cm. resulted, but there was no distinct separation of components (cf. ref. (9)).

3. Chromatography on diethylaminoethylcellulose (18). The DEAE-cellulose powder was first washed with 0.5N hydrochloric acid followed by 0.5N sodium hydroxide, before being made up into a slurry with water. The latter was then poured into a column (40 x 5 mm) which had a bed of glass-wool and silver sand, and allowed to settle under its own weight with a constant flow of water for four days. The column was then generated with 0.5M phosphate buffer (pH 6) and finally made ready for fractionation by passing 1000 ml of 0.05M phosphate buffer.

The initial runs with three nodules were carried out mainly to see if in fact a separation could be achieved. A solution of sample III

(electrodialysed, 360 mg in 10 ml of H_2O) was treated onto the column and allowed to be absorbed, before gradient elution first with phosphate buffer (0.05M - 0.25M) and then qith aq. sodium hydroxide (0.1M - 0.5M) was attempted. The average flow-rate was approx. 40 ml per hour. Fractions (40 ml) were screened by the phenol method (88). The elution pattern observed is shown in Fig. VI. The total recovery from the column was 331 mg, component A (117 mg. 35%) and component B (214 mg. 65%) contained 12.5% and 15.4% of uronic anhydride respectively.

Sample II (electrodialysed, 220 mg) gave an elution pattern similar to that shown in Fig. VII. Component A (66 mg. 34%) and component B (126 mg. 66%) had uronic anhydride contents of 12.4% and 14.9% respectively.

Sample IX (electrodialysed, 460 mg) similarly gave 180 mg (41%) of component A (uronic anhydride = 12.6%) and 258 mg (59%) of component B (uronic anhydride = 15.2%) (Fig. VIII).

From these three experiments it appeared that Acacia seyal was definitely heterogeneous, with at least two components which varied amongst other things in uronic acid content. The trail which was obtained in the first two runs (Fig. VI and VII) with the NaOH elution was thought to be due to perhaps more components which were overlapping. It was, therefore, decided to repeat the experiment (Fig. IX), using a step-wise elution with the phosphate buffer of molarities 0.1, 0.25 and 0.5 and aq. NaOH of molarities 0.1 and 0.5. Fractions were obtained with 0.25 and 0.5M phosphate buffer and with 0.1 and 0.5M sodium hydroxide. Each fraction was carefully dialysed in running water, further de-ionised on Amberlite 1R 45 (OH) and 1R 120 (H) to remove any inorganic matter, and finally taken down to small volume and freeze-dried. It was noticed at this stage that fractions obtained with aq. NaOH contained a certain amount of degraded cellulose

and therefore results obtained with the first three nodules concerning approx. amounts of polysaccharide material in fractions A and B were not strictly correct.

Each of the fractions was subjected to hydrolysis followed by a quantitative estimation of the amounts of monosaccharides present as well as the uronic acid anhydride content. At this stage the presence of xylose was detected in the sodium hydroxide fractions and since no trace had been detected on hydrolysis of the crude or purified gum, it was assumed that its presence was in some way connected with the degraded cellulose found earlier. The results are summarised below in Table VIII.

TABLE VIII

	Phosphate Buffer		Aq. Sodium Hydroxide	
	0.25 M	0.5 M	0.1 M	0.5 M
Fractions	(a)	(b)	(c)	(d)
Wt. in mg.	440	130	90	160
% Uronic A.A.	12.86	14.4	n.d.	15.1
% galactose	37	41	25	38
% arabinose	44	44	70	40
% rhamnose	4	1	none	3
% xylose	none	none	3	4

Total gum deposited on column = 910 mg.

" " recovered = 820 mg.

recovery = 90%.

Relative movements on electrophoresis of nodule IX and phosphate fractions I and II are shown in Fig. X.

DISCUSSION

The gum from A. seyal is similar to other Acacia gums in containing glucuronic acid, galactose, arabinose and rhamnose. The presence of acid labile residues and the marked decrease in viscosity detected on mild hydrolysis indicate that A. seyal probably further resembles Acacia gums in having a main chain, resistant to hydrolysis, to which is attached acid-labile side-chains. Of the Acacia gum studies to date, all have given negative optical rotations with the exception of A. karroo (72), to which must now be added A. seyal. The methoxyl content of the A. seyal nodules examined varied from 0.5 - 1.5%; only A. mollissima (75) has previously been reported to have a methoxyl content (0.35%). A methoxyl content of 1% has been found (23) to be significant in K.grandifolia gum.

The results presented in Tables IV, V and VI indicate that the inter-nodule variation in composition is greater than can be explained on the basis of possible analytical error. The variation is similar in extent to that previously found (19) for nodules of C. leonense gum. In Acacia gums, it is now well established that the removal of side-chains on mild hydrolysis to give "degraded gum" results in markedly decreased viscosity. It therefore seems reasonable to suggest that the differences (involving a variation of 100%) in the viscosity behaviour of the samples examined must reflect fine structural differences which involve the side-chains in some way. The kinetic differences on autohydrolysis (which normally cleaves only certain residues in the side-chains) and the variations in the percentage of uronic acid groups (which are normally present in side-chains or in chain-terminating positions) lend support to this suggestion.

The nodules examined were collected and authenticated by an expert on the identification of Acacia species. It may otherwise have been suggested that nodules V and VI (from their appearance), nodule VII (uronic acid content) and nodule VIII (viscosity) originated from some species other than A. seyal. Considering all the data for each nodule, however, there is little basis for doubting the authenticity of the samples. Taken jointly, the nitrogen content and the optical rotation of an Acacia gum are strongly indicative of its species: preliminary studies of other Sudanese Acacia species such as A. arabica, A. laeta, A. dealbata, A. drepanolobium, A. campylacantha (which have not been studied previously) have shown that the nitrogen content of A. seyal is characteristically low, and moreover, is not reduced by any of the methods of purification used. For some species of gum (cf. ref. (19)) significant reduction of the nitrogen content is given by purification processes (cf. ref. (16)). Our experiments on A. seyal and A. senegal, however, have confirmed the result recorded by Thomas and Murray (94) who could only reduce the nitrogen content of A. senegal from 0.36 to 0.33%. Frequently, the nitrogen content of plant gums is either not investigated (3, 72, 75) or recorded without comment (73, 77). Since Acacia gums form complexes with protein (16), it would now be of value to discover the origin of this residual nitrogen. Similar difficulties regarding the complete removal of nitrogen exist in investigations of starch and glycogen (cf. ref. (96)) and it has been suggested (98) that this nitrogen is present in residual traces of the enzyme systems involved in biosynthesis. The mechanism of gum formation is still far from clear (16), and further knowledge of the nature of the nitrogen content in plant gums would be of value in assessing the relative importance of the enzymic

polymerisation theory (8) in relation to the alternative theories (16, 99) that gum formation results from (a) normal plant metabolism, (b) pathological reactions to resist invading micro-organisms or to avoid loss of moisture (8).

Although it has been reported that the ash content of some species of gum can be eliminated (23, 92) by precipitation methods, our experiments with Acacia species have shown that their ash content cannot be reduced by more than about 50%, even after 4 re-precipitations. The results reported for A. seyal are typical in this respect. Electrodialysis is the most effective method of reducing the ash content to a very low value. Although an electrodialysis period of 6 hr. is much shorter than was used by earlier investigators (94), some degradation could conceivably have been caused. The viscosity characteristics of the nodules were therefore carefully investigated. As shown in Fig. II, the most viscous nodule (VIII) showed a marked decrease in viscosity on purification, although the other nodules were not affected to a comparable extent. In general, the purification methods studied do not appear to alter significantly the physical properties of the gum.

Fractionation of A. seyal gum on diethylaminoethyl-cellulose gave four components having uronic anhydride contents of 12.8%, 14.4%, n.d., and 15.1% respectively; the close similarity of the elution patterns suggested that different nodules contained the same components in slightly varying proportions. Conclusive evidence of heterogeneity is often difficult to achieve. Indeed, conflicting results may be given by different techniques; trypsin is electrophoretically heterogeneous, although only one component was evident on examination by ultracentrifuge (100). For gum arabic, chemical fractionation has been less successful than immunochemical experiments. Our failure to separate the components of A. seyal by electrophoresis (cf. ref. (9))

may therefore be explained by the fact that, in single nodules, the two components do not differ sufficiently in uronic acid content, upon which electrophoretic movement must depend to a large extent (cf. ref. (101)). Studies of the chemical structure of these two components are now in progress.

TABLE IV

Determinations on crude samples

	I	II	III	IV	V	VI	VII	VIII	IX
% moisture	13.5	13.6	13.3	14.3	11.0	11.0	16.1	15.9	11.4
	13.6	13.6	13.2	14.4	11.0	11.2	16.0	16.0	11.4
% ash	3.42	2.81	2.89	3.31	2.04	1.94	2.90	2.70	3.55
	3.38	2.98	2.94	3.33	2.10	2.10	2.94	2.80	3.61
% nitrogen	0.14	0.09	0.14	0.12	0.09	0.10	0.18	0.17	0.19
	0.15	0.10	0.14	0.13	0.10	0.10	0.19	0.18	0.19
% uronic anhydride	12.4	12.2	12.1	11.2	9.0	9.2	16.4	12.1*	11.6
	12.7	12.4	12.0	11.4	9.1	9.2	16.8	11.9	11.9
% methoxyl	n.d.	n.d.	0.60	0.72	1.36	1.53	1.0	0.82	0.55
Limiting Flow-time Number	8.7	8.7	12.7	14.7	8.2	9.8	15.3	19.0	15.6
$[\alpha]_D^{20}$	+52	n.d.	+56	+51	n.d.	+48	n.d.	+50	+44

* Zaidi 12.0, % OMe = 0.56. (89)

TABLE V

Determinations on samples purified by precipitation

	I	II	III	IV	V	VI	VII	VIII	IX
% ash	2.38	1.81	2.71	2.49	0.91	n.d.	n.d.	n.d.	2.48
	2.41	1.82	2.78	2.51	0.92				2.52
% nitrogen	0.14	0.09	0.13	0.16	0.07	0.10	0.18	0.17	0.20
	0.15	0.10	0.13	0.17	0.07	0.10	0.19	0.18	0.21
% uronic anhydride	13.1	12.8	12.5	12.8	10.4	10.9	16.6	12.9	12.4
	12.9	12.9	12.7	12.6	10.6	10.8	16.4	12.7	12.6
% methoxyl	n.d.	1.1	1.3	0.70	1.1	n.d.	n.d.	1.0	0.94
Limiting Flow-time Number	11.4	9.1	12.0	12.8	7.4	11.6	13.8	17.4	13.2
$[\alpha]_D^{20}$	+58	n.d.	+59	n.d.	n.d.	n.d.	n.d.	+64	n.d.

TABLE VI

Determinations of electrodialysed samples

	I	II	III	IV	V	VI	VII	VIII	XI
% ash	0.02	0.01	n.d.	n.d.	0.02	0.02	0.03	n.d.	0.05
	0.02	0.02			0.03	0.03	0.04		0.05
% nitrogen	0.15	0.11	0.16	0.17	0.10	0.11	0.19	0.17	0.17
	0.16	0.11	0.16	0.17	0.10	0.10	0.19	0.18	0.17
% uronic anhydride	13.6	13.0	13.5	13.8	12.1	12.5	16.8	13.4	13.8
	13.7	13.3	13.6	13.6	12.2	12.3	16.6	13.5	13.8
Limiting Flow-time Number	12.0	9.5	13.5	n.d.	7.0	12.4	13.6	14.2	n.d.

TABLE VII

Equivalent Weight Determinations on Acacia Seyal

Samples:	I		II		III		IV		V		VI		VII		VIII		IX	
	pH	Eq.wt.	pH	Eq.wt.	pH	Eq.wt.	pH	Eq.wt.	pH	Eq.wt.	pH	Eq.wt.	pH	Eq.wt.	pH	Eq.wt.	pH	Eq.wt.
Crude gum	4.49	8640	4.6	9400	4.6	11600	4.45	8570	4.41	4944	4.6	14833	4.65	13540	4.62	10500	4.5	11075
Precipitated gum	4.0	6749	4.0	6751	4.29	7601	4.0	6701	4.0	5085	3.9	5114	3.9	4991	3.8	4911	3.8	4451
Electrodialysed gum	2.7	1541	3.2	1520	3.14	1584	3.1	1546	2.81	1981	2.74	1620	2.7	n.d.	2.9	1530	2.8	1561

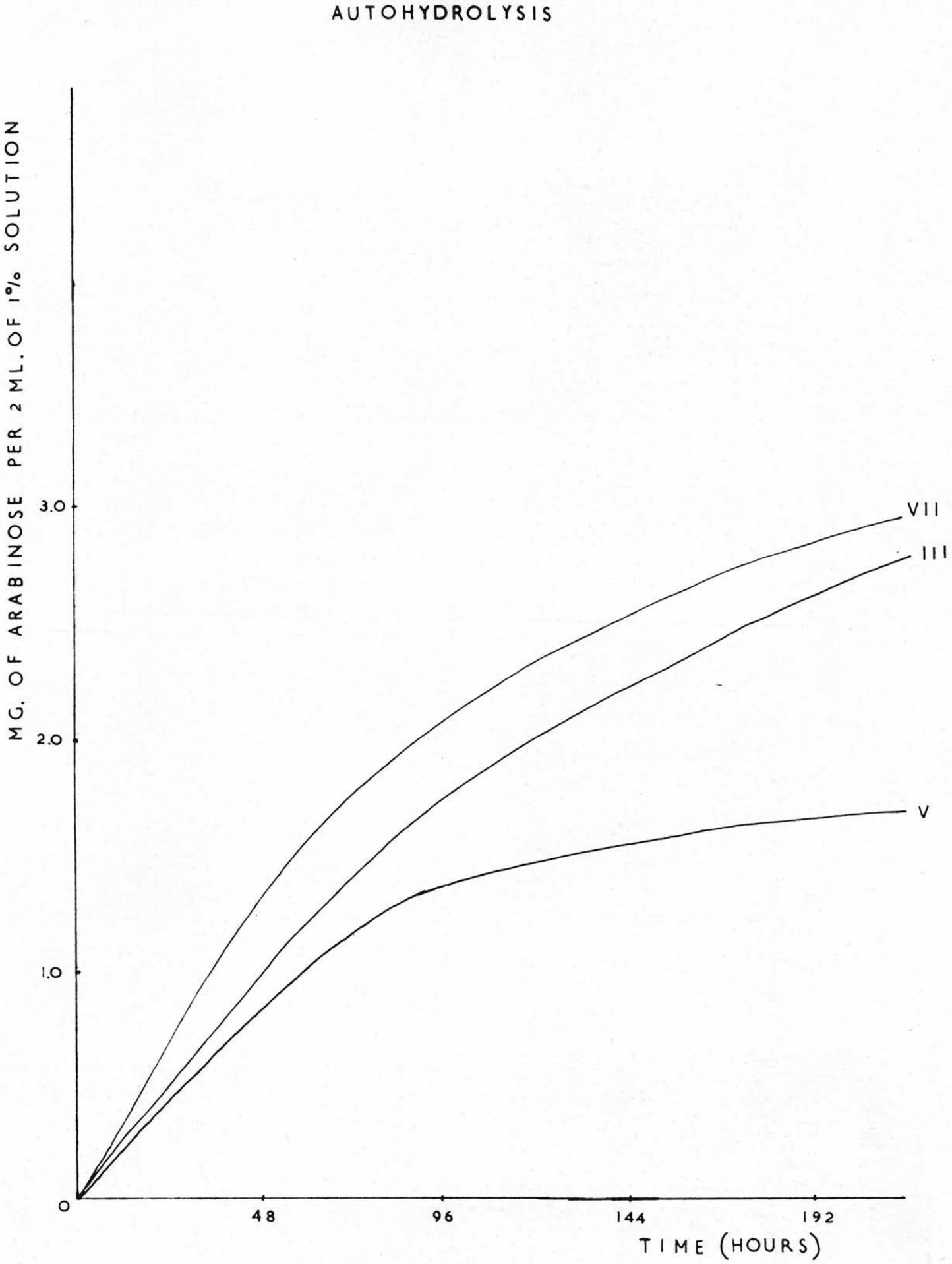

AUTOHYDROLYSIS
MG. OF ARABINOSE PER 2 ML. OF 1% SOLUTION
3.0
2.0
1.0
0
VII
III
V
48
96
144
192
TIME (HOURS)

FIG I

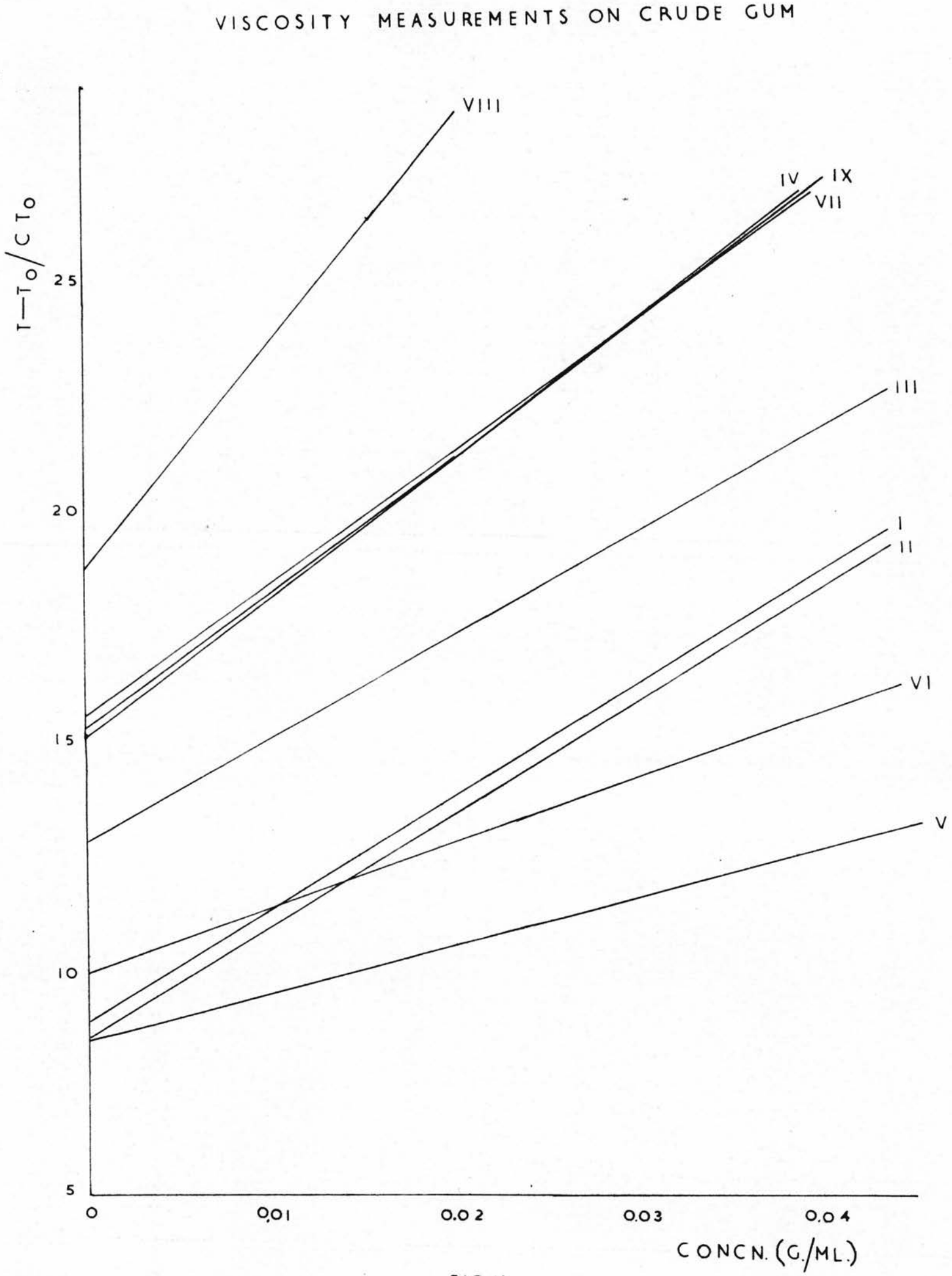

VISCOSITY MEASUREMENTS ON CRUDE GUM
T—T₀/C T₀
25
20
15
10
5
VIII
IV
IX
VII
III
I
II
VI
V
0
0.01
0.02
0.03
0.04
CONCN. (G./ML.)

FIG II

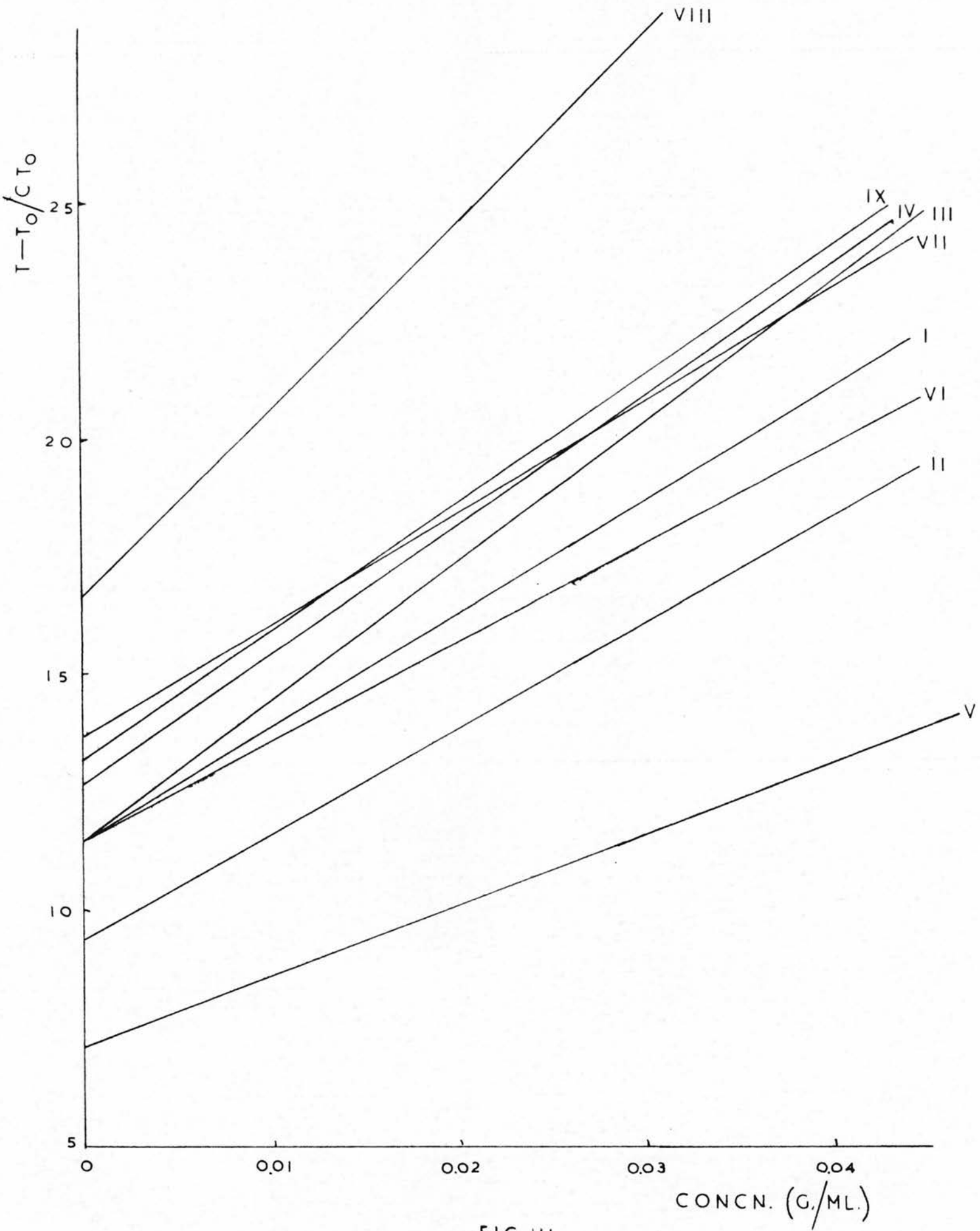

VISCOSITY MEASUREMENTS ON ALCOHOL PPTED. GUM
VIII
IX
IV
III
VII
I
VI
II
V
$T-T_0/CT_0$
25
20
15
10
5
0
0.01
0.02
0.03
0.04
CONCN. (G./ML.)

FIG III

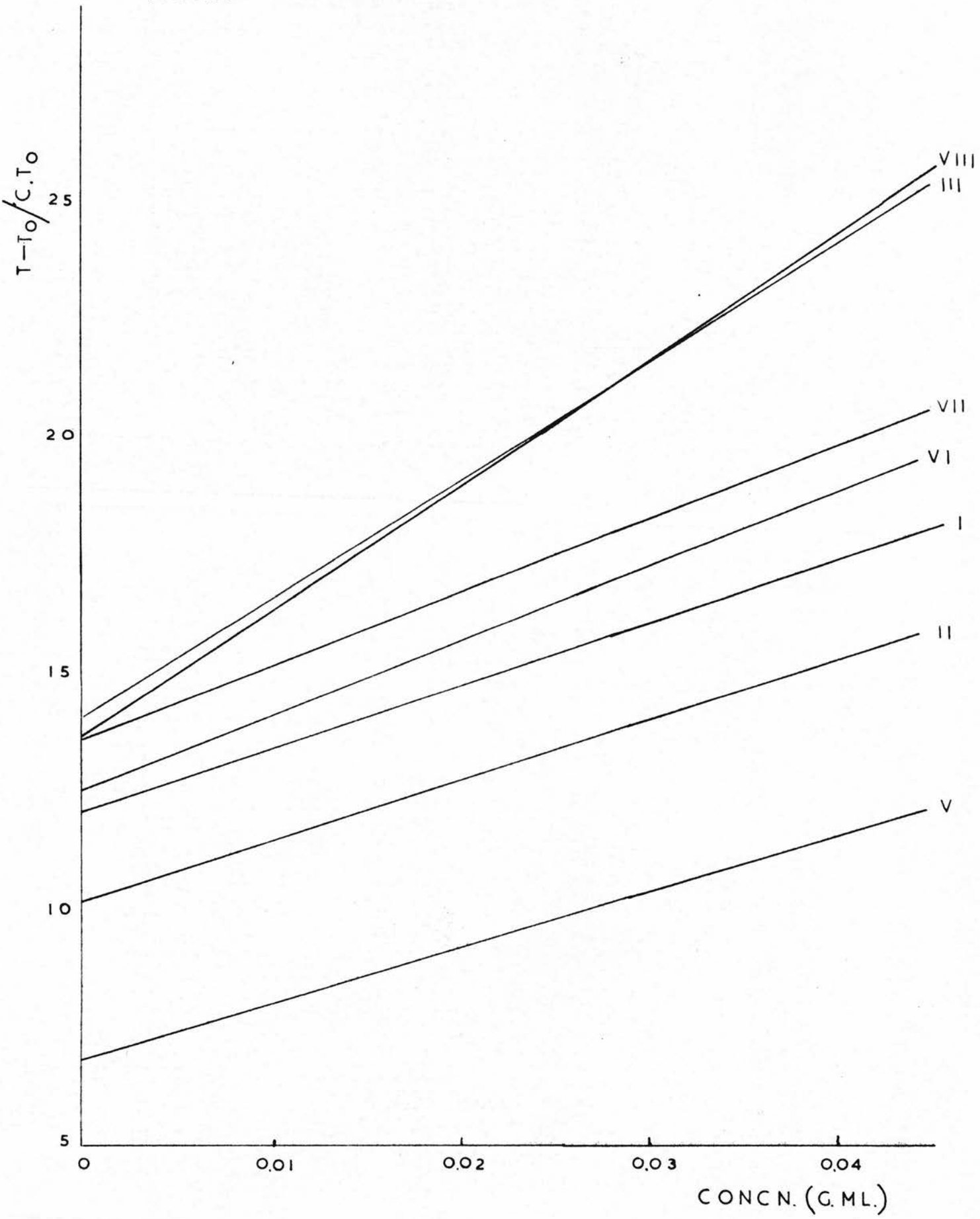

VISCOSITY MEASUREMENTS ON ELECTRODIALYSED GUM
T−T₀/C.T₀
25
20
15
10
5
VIII
III
VII
VI
I
II
V
0
0.01
0.02
0.03
0.04
CONCN. (G.ML.)

FIG IV

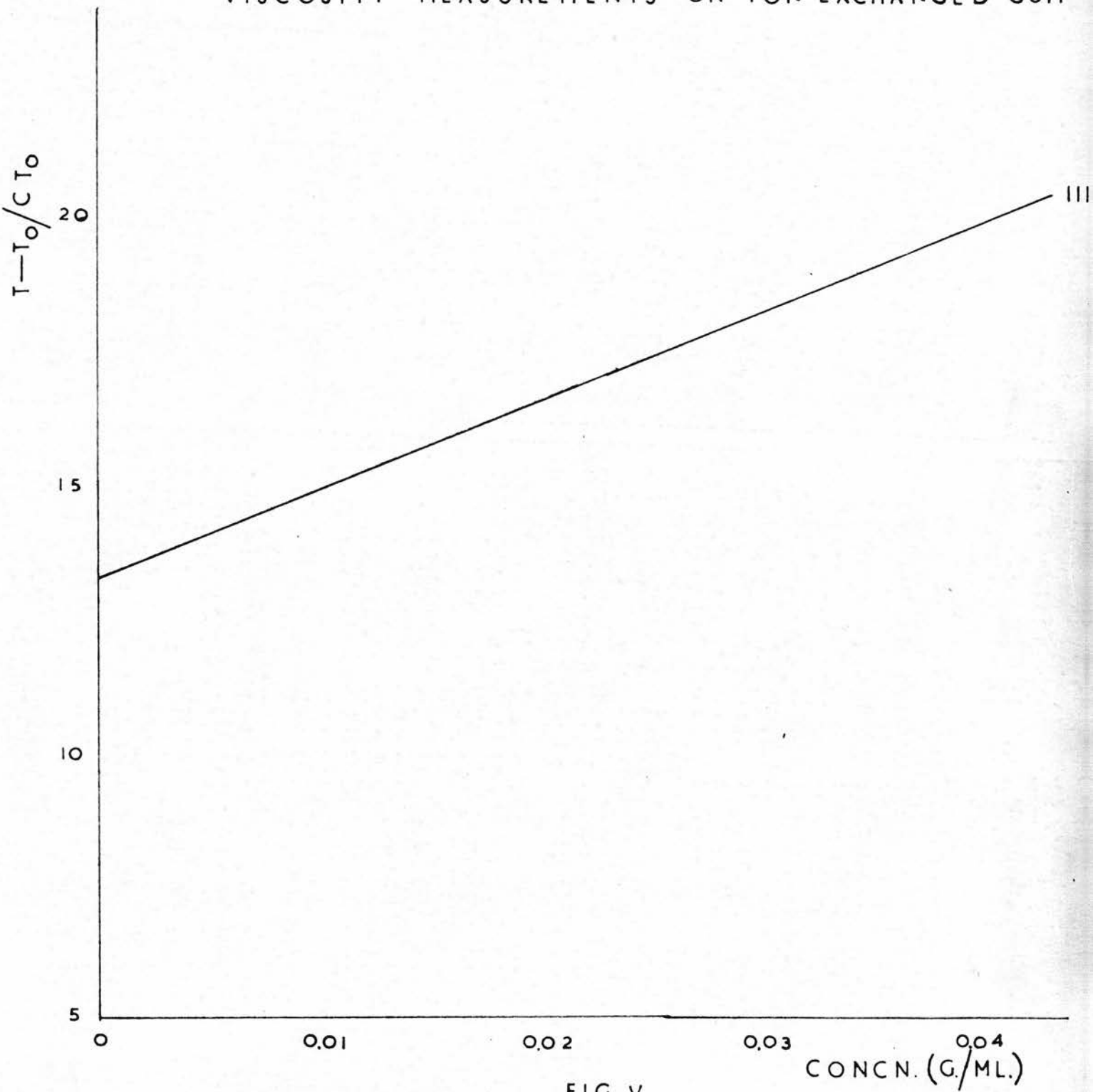

VISCOSITY MEASUREMENTS ON ION EXCHANGED GUM
T—T₀/C T₀
20
15
10
5
III
0
0.01
0.02
0.03
0.04
CONCN. (G./ML.)

FIG V

FRACTIONATION OF SAMPLE III ON DEAE CELLULOSE

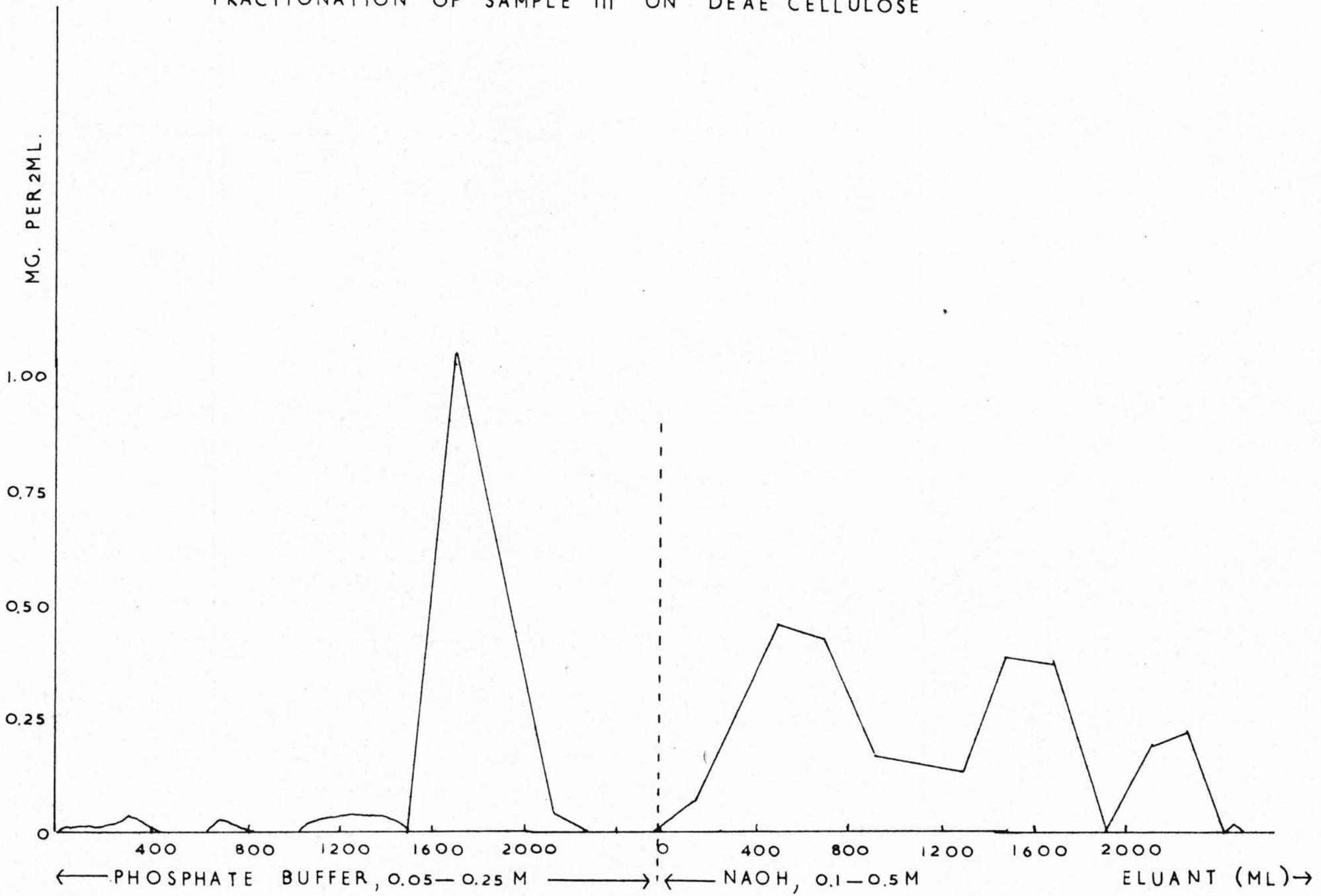


FIG VI

FRACTIONATION OF SAMPLE II ON DEAE CELLULOSE

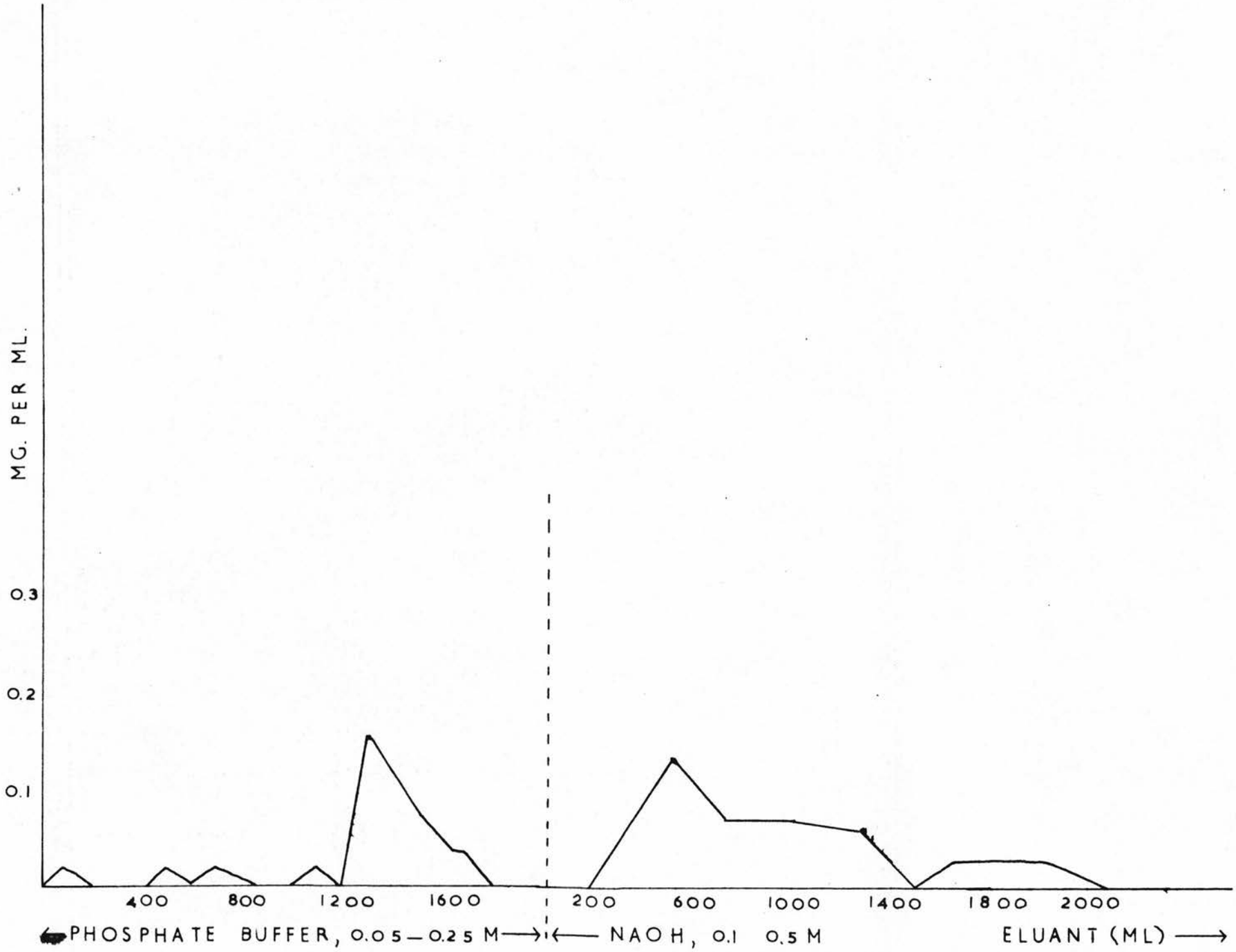


FIG VII

FRACTIONATION OF SAMPLE IX ON DEAE CELLULOSE

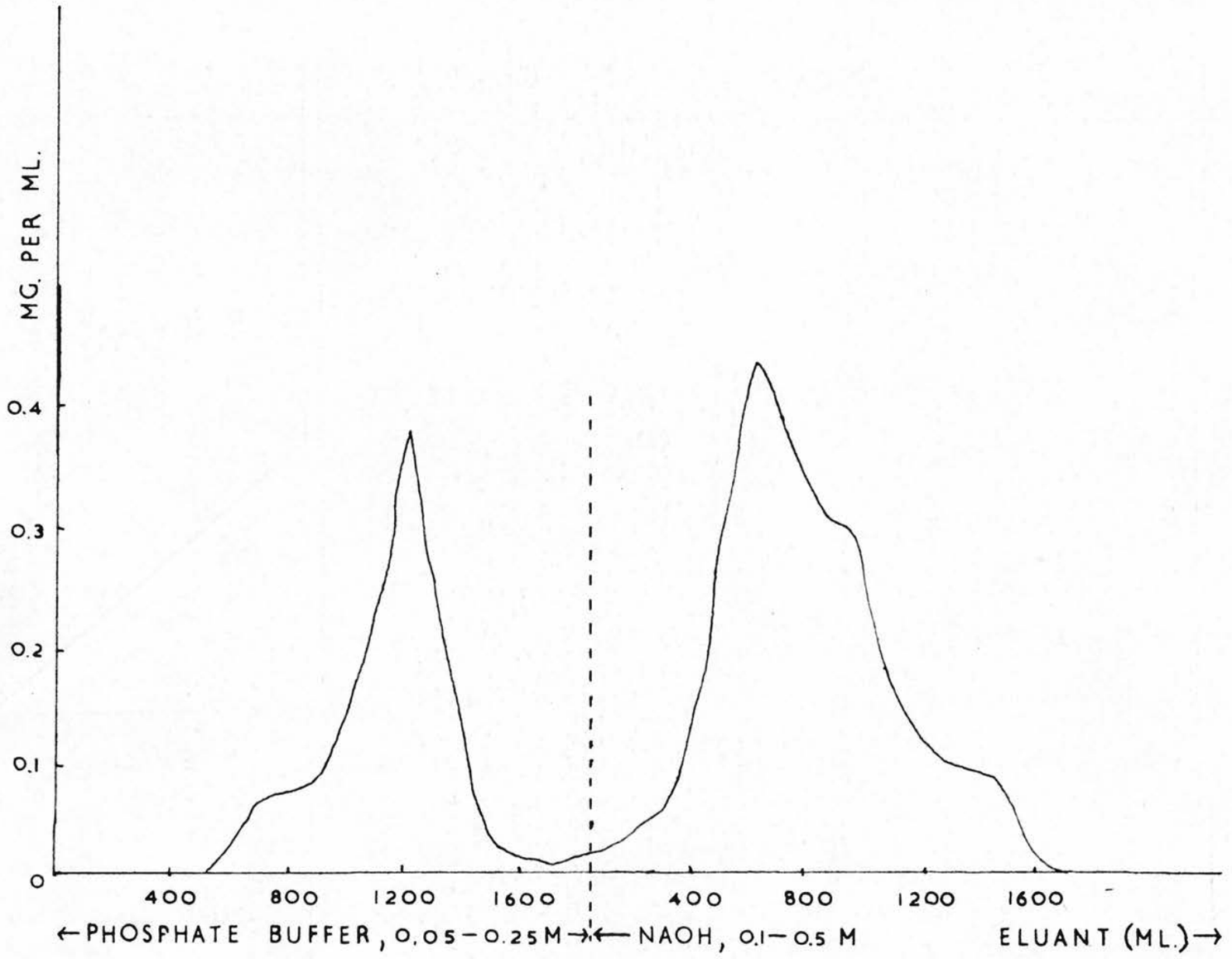


FIG VIII

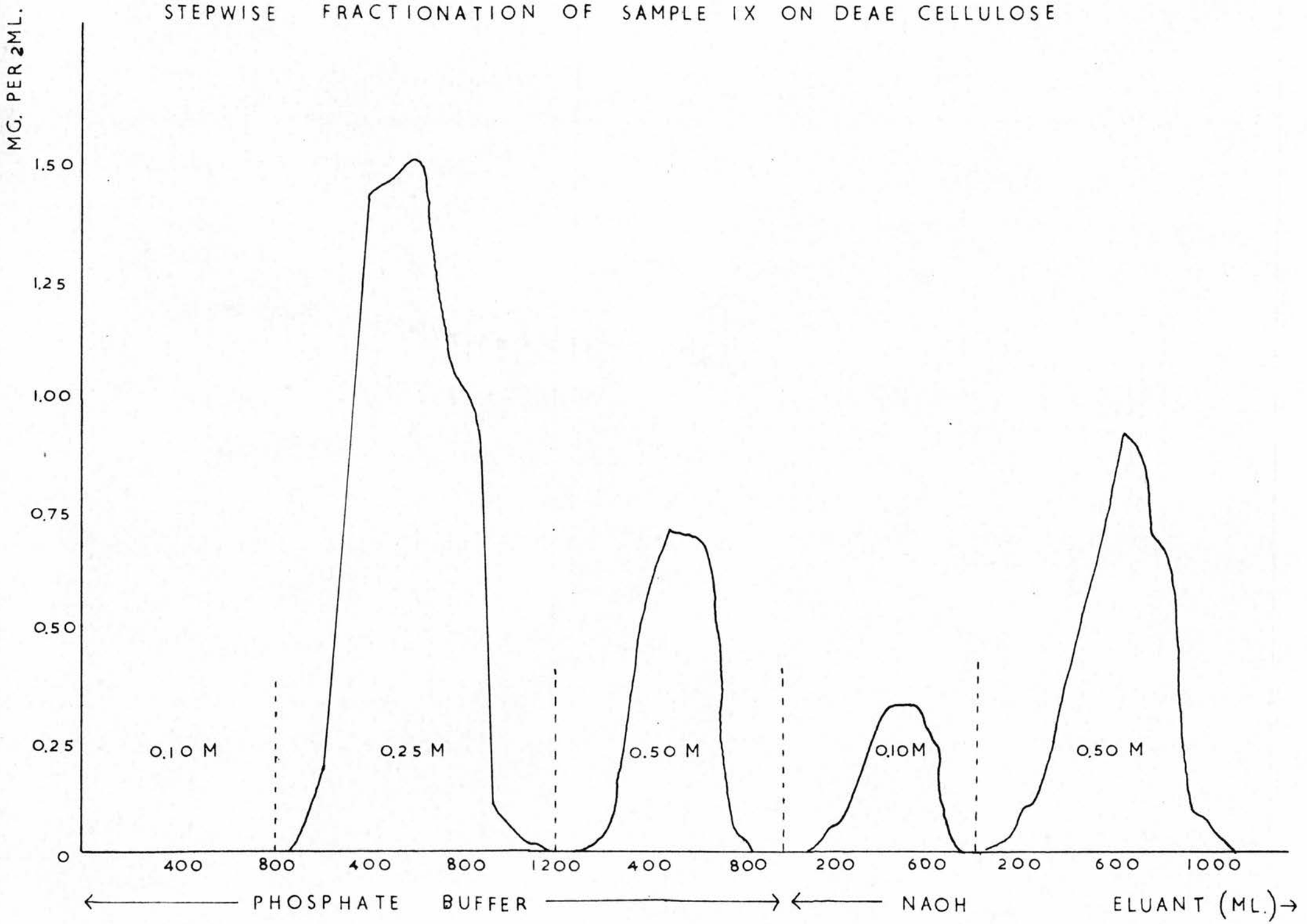

STEPWISE FRACTIONATION OF SAMPLE IX ON DEAE CELLULOSE
MG. PER 2ML.
1.50
1.25
1.00
0.75
0.50
0.25
0
0.10 M
0.25 M
0.50 M
0.10M
0.50 M
400
800
400
800
1200
400
800
200
600
200
600
1000
PHOSPHATE BUFFER
NAOH
ELUANT (ML.)

FIG. IX

ELECTROPHORESIS

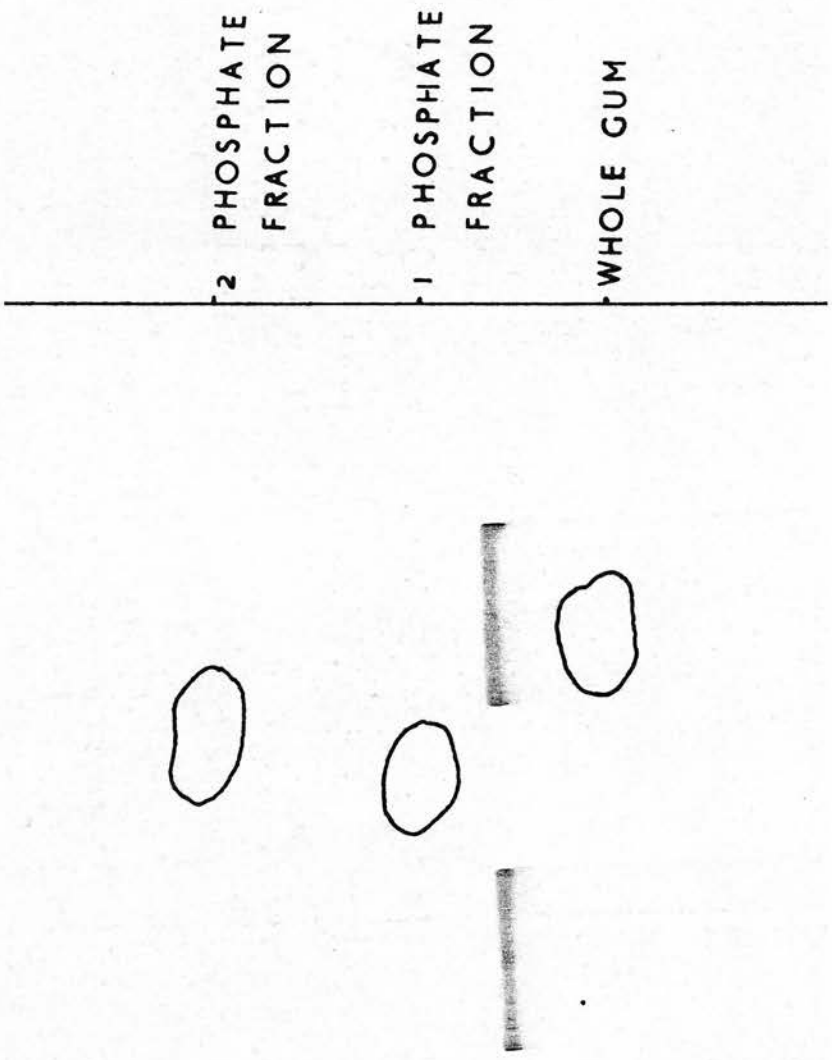


FIG X

SECTION III

Structural Studies on Acacia Seyal

(i) Graded hydrolyses.

(a) Sample IX (2 g) was subjected to acid hydrolysis (2N, H_2SO_4; 50 ml) at a temperature of 95°C. The solution became orange-red after 15 minutes and 2 ml aliquots were removed at the end of every half-hour up to 16 hours. Galactose, arabinose and rhamnose were present in all the aliquots; after 3 hours there was in addition a disaccharide which was characterised by chromatography methods as 3-O-β-D-galactosyl-L-arabinose.

(b) The strength of acid was reduced to 0.2N and it was found that after hydrolysis for half an hour only galactose and arabinose were present.

(c) On further dilution of the acid to 0.02N the hydrolysate after half an hour contained only arabinose. This, therefore, suggests that at least some of the arabinose is in the furanose form.

(ii) Partial acid hydrolysis

The gum (25 g) was heated in 0.01N H_2SO_4 (1500 ml) on a boiling water bath for 4 hours and the cooled solution was neutralised with Amberlite Resin IR 4B (OH). The solution was concentrated on a rotary evaporator at 30°C and poured into ethanol (4 vol); the precipitated degraded polysaccharide was separated and the supernatant liquid was concentrated to a syrup. Paper chromatography in solvent A and after 36 hours development indicated the presence of arabinose, an oligosaccharide and traces of galactose and rhamnose.

The degraded polysaccharide was rehydrolysed under the same conditions and yielded degraded gum A and a syrup. The combined syrups (10 g) were separated on a cellulose column (50 x 3.5 cm) using butan-1-ol half-saturated with water as eluant; four fractions were obtained (Table IX).

Degraded gum A was then subjected to further hydrolysis with 0.5N H_2SO_4 (1000 ml), for a period of one hour on a water bath. The cooled solution was neutralised to pH 5 with barium hydroxide and finally by barium carbonate. Inorganic material was removed at the centrifuge. The solution was concentrated, poured into ethanol (4 vol), the precipitated degraded polysaccharide was separated and the supernatant liquid concentrated to a syrup. The degraded polysaccharide was rehydrolysed under the same conditions giving degraded gum B (1 g) and a small amount of syrup. The combined syrups (ca. 10 g) were dissolved in a small amount of water and absorbed on a charcoal-celite column (32 x 5 cm).

The column was first developed with water to elute most of the monosaccharides and uronic acid residues, then with gradually increasing concentrations of aqueous alcohol. Fractions (20 ml) were collected every half hour (rate of flow of column 40 ml/hr), and every fifth fraction was

evaporated to small volume and examined chromatographically in solvents A, B and C. According to their contents the total number of fractions were combined to give the final seven (Table X).

TABLE IX

Fraction	Wt. in g	Contents
I	1.0	L-rhamnose and L-arabinose (trace)
II	2.14	L-arabinose and a pentose sugar R_{rha} 0.91 (trace)
III	6.14	L-arabinose, pentose, and an unidentified sugar R_{rha} 1.3 (trace)
IV	1.2	L-arabinose (trace) galactose and 3-O-β-arabinopyranosyl-L-arabinose.

The uronic acid content of degraded gum B was 19.7% and on hydrolysis with 2N-H_2SO_4 only glucuronic acid and galactose were obtained.

Examination of fractions II, III and IV was attempted in order to identify the unknown components which appeared on chromatographic examination of initial breakdown products of the partial hydrolysis. After a further separation of each fraction on thick chromatography paper in solvent A, elution with water and evaporation under reduced pressure, the two unknown components were obtained in very small quantities. Subfraction b of fraction II and subfraction b of fraction III were both chromatographically identical to D-ribose; both also gave $[\alpha]_D^{20}$ - 20° which further indicated D-ribose. Preparation of the p-bromophenyl hydrazine hydrochloride was, however, unsuccessful.

Subfraction c of fraction III was obtained in trace quantity only. Chromatographic examination indicated a mono- or di-methylated sugar with R_G 0.55 in solvents A and D. The presence of this sugar could account for the methoxyl content of Acacia seyal (approx. 1%).

TABLE X

Fraction	Eluant	Wt. in g.	Contents
A	water	9.16	D-galactose, L-arabinose, L-rhamnose, with traces of D-ribose and D-glucuronic acid.
B	2.5% EtOH	0.05	1-6-galactopyranosyl-D-galactose + trace of D-glucuronic acid.
C	2.5% EtOH	0.140	1-6-galactopyranosyl-D-galactose 1-3-galactosyl-L-arabinose traces of 1-3-arabinosyl-L-arabinose.
D	2.5% EtOH	0.216	1-3-arabinosyl-L-arabinose traces of 1-6-galactopyranosyl-D-galactose and 1-3-galactosyl-L-arabinose.
E	5.0% EtOH	0.008	1-6-galactobiose.
F	7.5% EtOH	0.224	1-3-galactobiose trace of a galactotetraose
G	10.0% EtOH	0.02	galactotetraose-L-arabinose.

Examination of the fractions and identification of the neutral and acidic oligosaccharides from partial hydrolysis (Table X).

Fraction A (9.16 g)

Since this fraction contained mainly monosaccharides which had already been identified in the preliminary investigation it was not examined further.

Fraction B (50 mg)

This fraction contained traces of glucuronic acid and a small quantity of the disaccharide 6-O-β-D-galactopyranosyl-D-galactose. The latter was separated from the former on 3 MM paper in solvent A, and was subsequently examined together with the larger quantity of the same disaccharide obtained from fraction C.

Fraction C 6-O-β-D-galactopyranosyl-D-galactose and 3-O-α-D-galactosyl-L-arabinose.

This fraction (160 mg) contained three components having R gal 0,23, 0.54 and 0.78 in solvent B and 0.26, 0.61 and 0.81 in solvent A. On separation on thick chromatography paper in solvent A, three fractions were obtained. After elution and subsequent evaporation to small volume, each fraction was examined as follows:-

Fraction Ca (115 mg) 6-O-β-D-galactosyl-D-galactose.

This fraction, having R gal = 0.26 and 0.23 in solvents A and B, $[\alpha]_D^{20}$ + 33° (C=0.15 in water) was chromatographically pure and identical to 6-O-β-D-galactosyl-D-galactose in the above solvents.

When a sample (10 mg) was hydrolysed with 1N-sulphuric acid at 95° for 4 hours, only galactose was detectable by paper chromatography. All attempts to crystallise the sugar were unsuccessful. Preparation of a phenylosazone of the sugar was also attempted, but it was not possible to obtain this in the crystalline form.

On methylation by Kuhn's method with subsequent methanolysis followed by hydrolysis, 2,3,4-tri-O-methyl-D-galactose and 2,3,4,6-tetra-O-methyl-D-galactose were identified by paper chromatography in solvents A, D and G. 2,3,4,6-tetra-O-methyl-D-galactose was confirmed by the preparation of 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine m.p. 191° (lit. 191°).

Fraction Cb (40 mg) 3-O-α-D-galactosyl-L-arabinose.

The $[\alpha]_D^{20}$ of the sugar was +156° (C = 0.5 in water). The syrupy sugar (10 mg) was hydrolysed with N-sulphuric acid for 4 hours at 95°; only galactose and arabinose were obtained. The remainder of the fraction (ca. 30 mg) was methylated first by Haworth's method with dimethyl sulphate and then by Kuhn's method. A fraction of the methylated product was examined by gas chromatography after methanolysis and was found to contain methyl-2,3,4,6-tri-O-methyl galactoside, methyl-2,4-di-O-methyl arabinoside and a trace of a tri-O-methyl galactoside.

The remainder of the methylated material was hydrolysed with hydrochloric acid (1N) on a boiling water bath, spotted on thick chromatography paper and separation effected with solvent D.

Fraction (a) (7 mg) R_G = 0.86

The sugar gave a single chromatographic spot which corresponded to 2,3,4,6-tetramethyl-D-galactose; this was confirmed by preparation of its anilide. (m.p. 199°).

Fraction (b) (6 mg) R_G = 0.65

This fraction was chromatographically pure and corresponded to 2,4-di-O-methyl-L-arabinose, but preparation of its anilide was not successful.

Fraction (c) R_G = 0.71

This was only a trace, and corresponded to 2,4,6-tri-O-methyl-D-galactose.

The high optical rotation of the original disaccharide obtained would seem to confirm the linkage to be an α one.

Fraction D 3-O-β-L-arabinopyranosyl-L-arabinose (main) + traces of 6-O-β-D-galactopyranosyl-D-galactose and 3-O-α-D-galactosyl-L-arabinose.

The separation of the components of this fraction (216 mg) was effected on 3 MM paper in solvent A with the main constituent (180 mg) having R gal = 0.86 in solvent A and $[\alpha]_D^{20}$ = +201° (C = 1.5 in water). Attempts to recrystallise the partially crystalline sugar from aqueous ethanol were unsuccessful.

A portion (150 mg) of the main fraction was methylated by Kuhn's method; on methanolysis of a portion (20 mg) and gas phase chromatographic examination, methyl-2,3,4-tri-O-methyl-L-arabinoside (T 1.03 in system b), methyl-2,4-di-O-methyl-L-arabinoside (T 2.27 in system b) and a small amount of methyl-2,5-di-O-methyl-L-arabinoside (T 1.83 in system b) were found. The remainder of the methylated disaccharide was hydrolysed with N-hydrochloric acid at 95° for 4 hours and the products fractionated on thick paper using solvent D. Two distinct fractions, identical chromatographically to 2,3,4-tri-O-methyl-L-arabinose and 2,4-di-O-methyl-L-arabinose were obtained. A faint greyish-black spot was also detected in solvents A, D and G. Derivatives of the two major products with aniline were attempted but no crystalline compounds could be obtained.

Fraction E

This fraction (8 mg) R gal 0.26 in solvent A and 0.23 in solvent B was chromatographically pure and identical to 6-O-β-D-galactosyl-D-galactose.

Fraction F 3-O-β-D-galactosyl-D-galactose + polygalactose-arabinose.

The components of this fraction (224 mg) which had R gal = 0.55 and 0.08 in solvent A, were separated on 3 MM paper.

Fraction Fa (15 mg)

This fraction had R gal = 0.08 in solvent A and $[\alpha]_D$ + 18° ± 2° (C = 1.5 in water). Complete hydrolysis with N-sulphuric acid at 95° for 8 hours gave only galactose with a trace of arabinose. On spotting the hydrolysate on thick paper and developing in solvent A, the two fractions were estimated by Somogyi's method, and the ratio of galactose to arabinose was found to be 10:1. This oligosaccharide, it was felt, therefore, was from the backbone of the gum with an arabinose residue attached at some point. In a recent study on Combretum leonense gum (21) an oligosaccharide tetragalactose-arabinose was isolated which exhibited an optical rotation $[\alpha]_D$ + 20° ± 2°.

Fraction Fb (200 mg) 3-O-β-D-galactosyl-D-galactose

This fraction, which had R gal = 0.55 in solvent A and $[\alpha]_D$ + 54°, was chromatographically pure and identical to 3-O-β-D-galactosyl-D-galactose in solvents A, C and E. Only galactose was obtained on hydrolysis with 2N H_2SO_4. Methylation, followed by methanolysis and hydrolysis, gave two methylated sugars. These were separated on 3 MM paper using solvent G, when the respective R_G values were 0.80 and 0.36.

Fraction Fba

This fraction (15 mg) was chromatographically pure and identical to 2,4,6-tri-O-methyl-D-galactose giving on demethylation only galactose. The aniline derivative failed to crystallise.

Fraction Fbb

This fraction (25 mg) was chromatographically pure and identical to 2,3,4,6-tetra-O-methyl-D-galactose which was identified by its aniline derivative (m.p. and mixed m.p. = 191°).

Finally, 3-O-β-D-galactosyl-D-galactose crystallised after standing for several weeks. A melting point of 168° and $[\alpha]_D = 58° \pm 2°$ (C = 1.0 in water) were obtained; the mixed melting point of the disaccharide with an authentic sample was undepressed.

A portion (1 g) of the degraded gum B (uronic anhydride 19%) obtained after hydrolysis of degraded gum A with 0.5N-H_2SO_4 was subjected to further hydrolysis with N-H_2SO_4 for 3 hours at 95°C. The solution, when cool, was neutralised with barium hydroxide and barium carbonate, de-ionised with Amberlite resin IR 120(H), and taken to a syrup. Chromatographic examination of the syrup in solvents A and B gave two distinct spots with Rgal values 1.0 and .14 and 1.0 and 0.41 respectively. Separation on 3MM paper in solvent A provided two fractions (i) and (ii):

Fraction (i) (195 mg) 6-O-β-D-glucuronosyl-D-galactose.

The syrup in water (C = 0.6) gave $[\alpha]_D^{20°} = +20.8$ and was chromatographically homogeneous. A portion (80 mg) was subjected to methanolysis, reduction with potassium borohydride and hydrolysis, whereupon, on chromatographic examination, galactose and glucose were obtained. Hydrolysis of a separate portion (40 mg) with 2N H_2SO_4 gave only glucuronic acid and galactose. The syrup was, furthermore, chromatographically identical to 6-O-β-D-glucuronosyl-D-galactose in solvent A, B and C. The low optimal rotation would indicate a β-linkage.

Fraction (ii)

This fraction (600 mg) was identical to D-galactose.

Methylation of Whole Gum

The gum acid (20 g) was dissolved in water (100 ml) and methylated in an atmosphere of nitrogen at 20°C. Methyl sulphate (250 ml) and sodium hydroxide solution (30% ; 600 ml) were added dropwise with stirring over a period of about 8 hours; stirring was continued for a further 12 hours. The mixture was then cooled in ice and almost neutralised with 5N H_2SO_4, then evaporated to a suitable volume (ca. 100 ml). The process of methylation was then repeated as above. On concentration of alk. solution after second methylation, there resulted a separation of the sod. salt of partially methylated gum in pale yellow plastic nodules. The latter was mechanically separated and remethylated, aqueous acetone being used as the solvent for the partially methylated sod. salt of the gum, using methyl sulphate (250 cc) and Na_2OH (30% ; 750 cc) at 35° over a period of 13 hours. The methylation was completed by heating the solution for 15 min at 65°C, whereby the excess of acetone was expelled and the Na salt of methylated gum separated on the surface of the methylation mixture as insoluble, pale yellow nodules. These were filtered off and the process of methylation was repeated a further three times. The crude sodium salt of methylated seyal gum acid was then dissolved in water, acidified with 5N H_2SO_4, whereupon the methylated acid precipitated as a white powder. The latter was then taken up in chloroform, dried over Na sulphate, and the solution concentrated under vacuum. When the concentrated chloroform solution was poured into excess of light petrol with stirring, the methylated gum acid was obtained as a white amorphous powder (5 g ; % OMe 38.4).

A small amount (500 mg) of the methylated gum was dissolved in water (15 ml), treated with silver carbonate, filtered, concentrated and

freeze-dried. This silver salt was dispersed in methyl iodide (10 ml) and methanol (5 ml). The mixture was refluxed in a dry flask fitted with a water condenser and calcium chloride tube. Silver oxide was added in four batches (each of 1 g) every four hours. The residue was filtered and extracted with hot chloroform. The combined filtrate and extract were concentrated to a small volume and the methylated gum was precipitated by addition of excess light petroleum (b.p. 60-80°). Yield of product = 400 mg ; ash = 1.84% ; OMe = 38.5%.

As the % OMe value did not increase appreciably the main bulk of the methylated gum (4.5 g) was taken as fully methylated.

Hydrolysis of the methylated gum

A trial hydrolysis was done on 100 mg. The gum was refluxed with methanolic hydrochloric acid (3% ; 5 ml) for 6 hours, treated with Amberlite resin IR 4B (OH), and evaporated to dryness. Approximately half of the syrup was examined by vapour-phase chromatography, when the presence of at least ten different methylated sugars was detected (Table XI). The remainder of the syrup was subjected to further hydrolysis with 0.5N hydrochloric acid for 6 hours. Examination by paper chromatography in solvents A, D and G confirmed the presence of the methylated sugars indicated by vapour-phase chromatography.

The rest of the methylated gum (2.6 g) was dissolved in sulphuric acid (2N, 100 ml), left to stand at room temperature for 3 days, diluted to double the volume with water, then heated on a water bath, when slight precipitation occurred. It was left, therefore, to stand at 40° for a further 3 days before being finally heated on a boiling water bath for 8 hours. Neutralisation of the solution was effected with barium hydroxide to pH 6, followed by barium carbonate. The solution was centrifuged and the filtrate evaporated to a small volume (ca. 25 ml). The solution was further filtered to remove barium sulphate (traces) and then concentrated to a syrup.

TABLE XI

Methyl glycosides of	T values in system ( )	
2,3,4-tri-O-methyl-L-rhamnose	0.47 (b)	0.47 (c)
2,3,5-tri-O-methyl-L-arabinose	0.56 & 0.71 (b)	0.6 (c)
2,3,4,6-tetra-O-methyl-D-galactose		1.6 (c)
2,5-di-O-methyl-L-arabinose	1.87 & 3.44 (b)	0.7, 1.01 & 1.06 (c)
2,3,4-tri-O-methyl-L-arabinose	1.05 (b)	0.85 (c)
2,3-di-O-methyl-L-arabinose	1.55 (b)	
2,4,6-tri-O-methyl-D-galactose	4.18 & 4.70 (b)	2.09 & 2.39 (c)
2,3,4-tri-O-methyl-D-glucuronic acid	2.53 & 3.25 (b)	1.78 & 2.26 (c)
2,4-di-O-methyl-D-galactose	3.81 & 4.52 (c)	
2,3,6-tri-O-methyl-D-galactose	1.61 (c) ?	

Separation of acidic and neutral methylated sugars.

The syrup was placed on a cellulose column (75 x 3.5 cm) and the column was eluted with butan-1-ol saturated with water.

Elution of sugars was complete after about 3 litres of eluant had passed through the column. The column was then washed with water until again no more carbohydrate material was detected. On examination of the two concentrated fractions, the former was found to contain only neutral methylated sugars whilst the latter, after de-ionising a portion with Amberlite resin IR 120 (H), was found to contain acidic components which streaked in solvent A and G.

Examination of neutral methylated sugars (Table XII)

The neutral methylated sugars (2.4 g) were separated by chromatography on a cellulose column (75 x 3.5 cm). The column was successively eluted with the following solvents:

(a) Light petroleum (b.p. -100-120°) : butan-1-ol (7 : 3 saturated with water)

(b) " " " : " (1 : 1 ")

(c) Butan-1-ol half saturated with water.

(d) Water.

TABLE XII

Fraction	R_G	Colour	Wt.mg	Contents
1	1.01	Green-grey	100	2,3,4-tri-O-methyl-L-rhamnose
2	0.98	Black, red in U.V.	460	2,3,5-tri-O-methyl-L-arabinose
	0.88	Reddish-brown		2,3,4,6-tetra-O-methyl-D-galactose
3	0.88	Reddish-brown	540	2,3,4,6-tetra-O-methyl-D-galactose
	0.80	Black, red in U.V.		2,5-di-O-methyl-L-arabinose
	0.78	Pink-brown		2,3,4-tri-O-methyl-L-arabinose
4	0.80	Black, red in U.V.	153	2,5-di-O-methyl-L-arabinose
	0.78	Pink-brown		2,3,4-tri-O-methyl-L-arabinose
	0.75	Reddish-brown		2,4,6-tri-O-methyl-D-galactose
5	0.75	Reddish-brown	145	2,4,6-tri-O-methyl-D-galactose
6	0.71	Reddish-brown	40	Tri-O-methyl-D-galactose
7	0.71	Reddish-brown	65	Tri-O-methyl-D-galactose
	0.59	Pink-brown		2,3-di-O-methyl-L-arabinose
8	0.54	Reddish-brown	340	2,4-di-O-methyl-D-galactose
9	0.54	Reddish-brown	156	2,4-di-O-methyl-D-galactose
	0.40	Khaki		L-rhamnose (trace)
	0.31	Brown		2-O-methyl-D-galactose (trace)
10	0.20	Brown	30	3-O-methyl-D-galactose ?
	.18	Pink		Arabinose (trace)
	.16	Brown		Galactose (trace)
11 (water)		Reddish-pink streak	40	Acidic methylated sugars and monosaccharides.

Total weight recovered = 2.09 g (89%)

Examination of fractions

Fraction I (100 mg) 2,3,4-tri-O-methyl-L-rhamnose

This fraction (100 mg) Rg = 1.01 and $[\alpha]_D$ = +26° (C = 0.5) was chromatographically identical to 2,3,4-tri-O-methyl-L-rhamnose in solvents A, D and G and was characterised by conversion to the aniline derivative, which when recrystallised from light petroleum gave a m.p. 113°-115° and was undepressed on admixture with authentic 2,3,4-tri-O-methyl-N-phenyl-D-rhamnosylamine. Demethylation gave only rhamnose.

Fraction II (460 mg) 2,3,5-tri-O-methyl-L-arabinose (main) and 2,3,4,6-tetra-O-methyl-D-galactose.

Chromatography of this fraction (460 mg) in solvent D showed two spots R_G 0.98 and 0.88, touching each other. In solvent A it gave a black streak corresponding to the trimethylarabinose and the top of the streak was pink. Chromatography in solvent G, however, showed two distinct spots; the pink spot corresponding to 2,3,4,6-tetra-O-methyl-D-galactose separated well from the black streak. The syrup was separated into two fractions on thick paper using solvent system G.

Subfraction a

This fraction (120 mg) was chromatographically identical to 2,3,4,6-tetra-O-methyl-D-galactose. The sugar was finally characterised by preparing the aniline derivative which gave a melting point of 199° which was undepressed when mixed with an authentic sample of 2,3,4,6-tetra-O-methyl-N-phenyl-D-galactosylamine. On demethylation only galactose was obtained.

Subfraction b

Chromatography of this fraction (320 mg) R_G = 0.98 and $[\alpha]_D^{20}$ - 38° (C = 1.0 in water) showed it to be homogeneous and identical to 2,3,5-tri-O-methyl-L-arabinose. With aniline oxalate it gave a characteristic black stain (red under u.v. light). It was characterised by conversion to the crystalline amide of the aldonic acid, which on recrystallisation from ethyl acetate gave a melting point of 135° (lit. 130°-138°). On demethylation only arabinose was obtained.

Fraction III 2,5-di-O-methyl-L-arabinose (main) 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-arabinose.

This fraction (540 mg) on chromatography in solvent A showed one spot which was black but which was pink at the top and bottom. However, chromatography in solvent G gave three distinct spots, corresponding to 2,3,4,6-tetra-O-methyl-D-galactose, 2,5-di-O-methyl-L-arabinose (black spot) and 2,3,4-tri-O-methyl-L-arabinose. The syrup was separated into three fractions on thick paper using solvent G.

Subfraction a R_G 0.68 and $[\alpha]_D^{20}$ + 108° (C = 0.2 in water)

This fraction (80 mg) was chromatographically identical to 2,3,4-tri-O-methyl-L-arabinose and gave a pink colouration with aniline oxalate which suggested that the sugar was in the pyranose ring form. All attempts to prepare the amide of the aldonic acid were unsuccessful.

Subfraction b R_G 0.80 and $[\alpha]_D$ - 2° (C = 0.4 in water).

On chromatographic examination in solvents A, D and G, this fraction (380 mg) was homogeneous and identical to 2,5-di-O-methyl-L-arabinose

giving a black colouration with aniline oxalate which is usually associated with the furanose ring form. On preparation of the amide of the aldonic acid, a m.p. of 127° was obtained which was undepressed on admixture with an authentic sample of 2,5-di-O-methyl-L-arabinamide. On demethylation only arabinose was obtained.

Subfraction c R_G 0.88 and $[\alpha]_D$ + 114° (C = 0.5)

This fraction (70 mg) was identified chromatographically and by an aniline derivative as 2,3,4,6-tetra-O-methyl-D-galactose in a similar manner to that described for subfraction a of fraction II. Demethylation gave galactose with a trace of arabinose.

Fraction IV 2,4,6-tri-O-methyl-D-galactose
2,3,4-tri-O-methyl-L-arabinose
2,5-di-O-methyl-L-arabinose

Chromatographic examination of this fraction (153 mg) revealed three spots with R_G values 0.75, 0.78 and 0.80 in solvent D. Examination in solvents A and B showed up only one spot which had a black lower edging but which was otherwise reddish-brown in colour. A very clear separation of three components was, however, obtained in solvent G when the respective R_G values were 0.36, 0.68 and 0.78 and which corresponded to 2,4,6-tri-O-methyl-D-galactose, 2,3,4-tri-O-methyl-L-arabinose and 2,5-di-O-methyl-L-arabinose respectively.

The syrup was separated on thick paper in solvent G.

Subfraction a R_G 0.36 and $[\alpha]_D$ + 84° (C = 1.0 in water)

This fraction (120 mg) was chromatographically pure and identical to 2,4,6-tri-O-methyl-D-galactose. The aniline derivative gave needle shaped crystals when recrystallised from acetone : ether : petroleum (1 : 1 : 1) and

a m.p. 165° (lit. 165°). On demethylation only galactose was obtained.

Subfraction b R_G 0.68

This fraction (10 mg) was identical chromatographically to subfraction a of fraction III, namely, 2,3,4-tri-O-methyl-L-arabinose, but due to the small amount present no derivatives were attempted.

Subfraction c R_G 0.78

This fraction (15 mg) was identical to 2,5-di-O-methyl-L-arabinose as in subfraction b of fraction III. On demethylation, only arabinose was obtained.

Fraction V 2,4,6-tri-O-methyl-D-galactose

R_G 0.75 $[\alpha]_D$ + 83° (C = 1.0)

Chromatographic examination of this fraction (145 mg) in solvents A, D and G revealed only one spot, which was identical to 2,4,6-tri-O-methyl-D-galactose. The aniline derivative was obtained with m.p. 164° and when mixed with authentic sample of 2,4,6-tri-O-methyl-N-phenyl-galactosylanine there was no depression of melting point. On demethylation, only galactose was obtained.

Fraction VI Tri-O-methyl-D-galactose

This fraction (40 mg), R_G 0.71, when examined chromatographically was pure and corresponded to 2,3,4-tri-O-methyl-D-galactose in solvent systems A and G. In solvents B and D it could not be distinguished from 2,4,6-tri-O-methyl-D-galactose. An aniline derivative was attempted but failed to crystallise. On demethylation only galactose was obtained.

Fraction VII

Chromatographic examination of this fraction revealed two spots in solvents A and D whilst in solvent G only one spot was observed. The main component which had R_G 0.59 (pink-brown) was thought to be a di-O-methyl-L-arbopyranose whilst the smaller component corresponded chromatographically to 2,3,4-tri-O-methyl-D-galactose. Demethylation of the mixture gave arabinose and a small amount of galactose. Separation of this fraction was effected on 3 MM paper in solvent D.

Subfraction a R_G 0.59 $[\alpha]_D^{20} = +83^\circ$ (C = 0.5 in water)

This fraction (35 mg) was chromatographically pure and identical to 2,3-di-O-methyl-L-arabinose. The amide of the aldonic acid was prepared but failed to crystallise.

Subfraction b R_G 0.70

This fraction (19 mg) was chromatographically identical to 2,3,4-tri-O-methyl-D-galactose and on demethylation only galactose was obtained.

Fraction VIII 2,4-di-O-methyl-D-galactose (340 mg) R_G 0.54, $[\alpha]_D^{20} = +88^\circ$ (C = 1.0 in water)

Examination of this fraction in solvents A, D and G showed only one spot which corresponded to 2,4-di-O-methyl-D-galactose. The sugar crystallised on standing, and on recrystallisation from acetone containing 1% water, needle-shaped crystals m.p. $97^\circ - 98^\circ$ were obtained which on admixture with authentic 2,4-di-O-methyl-D-galactose (m.p. 101°) did not

undergo depression. Chromatographic examination of periodate oxidation products revealed only unchanged 2,4-di-O-methyl-D-galactose and no other spots. Demethylation gave only galactose.

Fraction IX. 2-O-methyl-D-galactose
rhamnose (trace)
2,4-di-O-methyl-D-galactose

Chromatographic examination in solvents A and D showed up three distinct spots with R_G values 0.54, 0.40 and 0.31, with the latter two being very faint.

Separation was effected on 3 MM paper when three distinct fractions were obtained.

Subfraction a (15 mg) R_G 0.31

This sugar was chromatographically pure and identical to 2-O-methyl-D-galactose, and on demethylation gave only galactose.

Subfraction b (5 mg) R_G 0.40

Present in trace quantity, chromatographically identical to rhamnose, this fraction was not examined further.

Subfraction c (130 mg) R_G 0.54

This fraction was easily crystallised and chromatographically was identical to 2,4-di-O-methyl-D-galactose. Demethylation gave only galactose.

Fraction X

This fraction (30 mg) comprised mainly the monosaccharides L-arabinose and D-galactose together with a monomethylated galactose (demethylation gave galactose) present in very small quantity. Chromatographically in solvents A, B and D it corresponded to 3-O-methyl-D-galactose, having R_G = 0.2 in solvent D.

Fraction XI L-arabinose
D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid

This fraction (40 mg) could not be separated into individual spots in solvents A, B, D or G, but in solvent C three distinct spots were obtained; on separation of the latter on 3 MM paper, subfractions a and b were found to be galactose and arabinose.

Subfraction c R_G 0.41 2,3,4-tri-O-methyl-D-glucuronic acid

This fraction (20 mg) which was obviously an acidic component was converted to the methyl ester methyl glycoside with dry methanolic hydrochloric acid, reduced with lithium aluminium hydride in tetrahydrofuran and hydrolysed with hydrochloric acid. Chromatographic examination of the product in solvents C and D showed only one spot (brown) R_G = 0.83 which corresponded to 2,3,4-tri-O-methyl-D-glucose.

Examination of the Acidic Methylated Sugars

The barium salts of the acidic methylated sugars obtained from the preliminary separation (468 mg) were dissolved in water and treated with Amberlite resin IR 120 (H). After concentration, a small part of the brown syrup obtained was examined chromatographically. Five spots were obtained, the first two near the top of the paper, whilst the latter three, only in trace quantity, corresponded to 2,4-di-O-methyl-D-galactose, arabinose and rhamnose.

Separation of the first two fractions from the latter three was effected on 3 MM paper in solvent A. These two fractions were then concentrated to a syrup and refluxed with 2% methanolic hydrochloric acid (25 ml) for 6 hours, neutralised with silver carbonate and concentrated to a syrup. The product was dissolved in anhydrous tetrahydrofuran, lithium aluminium hydride (300 mg) was added, and the mixture was refluxed for one hour. More lithium aluminium hydride was then added and the mixture was refluxed for another hour. Excess hydride was removed by addition of ethyl acetate and water alternately. The mixture was filtered, the residue washed with hot acetone and chloroform, and the combined filtrate and washings taken to dryness. The product was then extracted with dry chloroform, filtered, and taken to dryness again. This reduced material was finally hydrolysed with 1N-hydrochloric acid (20 ml) for 6 hours, the solution was neutralised with silver carbonate, de-ionised, and the products examined chromatographically.

Fraction I (15 mg) $R_G = 1.1$

This minor fraction was chromatographically pure and identical to D-glucose. It could have arisen as a reduction product of D-glucuronic acid which escaped methylation or was demethylated in the early part of the methylation studies.

Fraction II (155 mg) $R_G = 0.84$

This fraction was chromatographically identical to 2,3,4-tri-O-methyl-D-glucose and was finally identified by preparation of the aniline derivative which gave a m.p. of 132°, and which, when mixed with an authentic sample of 2,3,4-tri-O-methyl-N-phenyl-D-glucosylamine, was not depressed.

Methylation of Degraded Gum B (p.61)

A sample of degraded gum B (300 mg) was subjected to methylation by Kuhn's method and after three successive methylations, the methylated product was isolated by extraction with chloroform. Evaporation of this solvent followed by methanolysis and subsequent hydrolysis provided a mixture of methylated sugars (35 mg) which could only be examined by paper chromatography. Using solvent systems A, C, D, E and G and standard methylated sugars, the following methylated sugars were identified:

2,3,4-tri-O-methyl-D-glucuronic acid (heavy spot)

2,4-di-O-methyl-D-galactose (heavy spot)

2,3,4-tri-O-methyl-D-galactose (heavy spot)

2,4,6-tri-O-methyl-D-galactose

2,3,4,6-tetra-O-methyl-D-galactose (trace)

(iv) Smith Degradation of Whole Gum

(a) Trial Smith degradation

The gum (1 g) was dissolved in water (20 ml) and to it freshly prepared sodium metaperiodate solution (75 ml; 0.1884N) was added; the solution was made up to 100 ml. In another flask 75 ml of the same periodate solution was diluted to 100 ml with distilled water. In a third flask, 75 ml of the periodate solution was treated with excess ethylene glycol and the mixture diluted to 100 ml to give a sodium iodate solution of the same molarity as the above two solutions. All three solutions were shaken and kept in the dark at room temperature.

After certain intervals of time a 1 ml aliquot was removed from each solution, diluted 2000 times and the optical density of the resulting solution measured in the Unicam spectrophotometer at 222.5 mμ (69). From these results the consumption of periodate per sugar unit after various intervals of time was calculated. The results are tabulated below.

Time (hr.)	2	5	11	24	30
Moles of periodate consumed/sugar unit	0.49	0.64	0.69	0.70	0.71

After 36 hours the oxidation was stopped by addition of ethylene glycol (2 ml) and the solution was dialysed against tap water for three days. The dialysate was concentrated to a small volume (25 ml), potassium borohydride (200 mg) was added and the solution was allowed to stand for six hours. At the end of this period, a further portion (200 mg) of potassium borohydride was added and the solution was allowed to stand overnight at room temperature. The solution was treated with Amberlite

resins IR 120(H) and IR 45 (OH), concentrated to a syrup; methanol was then added and the solution taken to dryness. This was repeated three times in order to remove all borate ions.

The residue was dissolved in N-sulphuric acid and allowed to stand at room temperature. After 3 hours the solution was neutralised by addition of barium hydroxide followed by barium carbonate. The neutralised solution was treated with Amberlite resin IR 120(H), and concentrated to a small volume. The degraded gum (100 mg), precipitated by pouring the concentrated solution into excess ethanol, was centrifuged off and was washed with ethanol followed by methanol. The combined centrifugate and washings were taken to dryness to give the low molecular weight material(200 mg).

On chromatographic examination in solvents A and H, the latter was found to contain glycerol and a trace of arabinose. The possibility of threitol and/or erythritol being present could not be discounted as the mobility of these two sugar alcohols in solvents A and H were similar to glycerol. On hydrolysis with N-sulphuric acid for 6 hours, this material yielded arabinose, galactose and rhamnose in addition to the above sugar alcohols.

A sample of the degraded gum on hydrolysis with 2N H_2SO_4 yielded galactose and arabinose with a trace of glucose and glucuronic acid.

(b) Large scale Smith degradation

The gum (14 g) was dissolved in water (500 ml), sodium periodate solution (45 g in 500 ml) was added and the mixture shaken and allowed to stand in the dark at room temperature. The change in periodate concentration was followed spectrophotometrically (69) and no further uptake of the oxidant was observed after 32 hours. At this stage excess periodate

was destroyed by addition of ethylene glycol and the reaction mixture was dialysed against tap water for three days.

The dialysate was concentrated to a small volume and reduction to the polyalcohol effected by addition of potassium borohydride (3 g); the mixture was kept at room temperature for two days. The solution was then treated with Amberlite resin IR 120 and IR 45, concentrated to small volume and poured into excess ethanol containing 5% light petroleum. The polyalcohol separated in a colloidal form and could not be isolated. Hence the solution was taken to dryness and the residue was repeatedly evaporated with methanol to remove borate ions.

The polyalcohol (10 g) thus obtained was dissolved in N-sulphuric acid (150 ml) and allowed to stand at room temperature. The hydrolysis was stopped after 3 hours by neutralisation with barium hydroxide and barium carbonate. The neutralised solution was filtered, treated with Amberlite resin IR 120, concentrated to a small volume and poured into excess ethanol. The degraded polysaccharide C separated out as a "gummy" precipitate. This precipitate was washed several times with ethanol before finally being dissolved in water, dialysed against tap water for one day and freeze-dried (8.2 g). The centrifugate and alcohol washings were taken to dryness to give low molecular weight materials (2.6 g).

Chromatographic examination of the latter revealed exactly the same components as the corresponding mixture obtained in the trial experiment described earlier, namely, glycerol, rhamnose, galactose and arabinose. These were not further investigated since little structural data could be obtained from these reaction products. Examination of the polysaccharide C was carried out as this could yield important information concerning the "backbone" of the gum.

(c) Examination of Polysaccharide C

(i) The degraded polysaccharide C was found (89) to have a uronic acid content of 4.6% and on total hydrolysis of a fraction (100 mg) with 2N-sulphuric acid at 95°C, only galactose, arabinose and a faint spot corresponding to D-glucuronic acid were found.

(ii) The remainder of the degraded polysaccharide C (7.5 g) was methylated with dimethyl sulphate and 30% aq. sodium hydroxide, six such operations being carried out. It was found that the methoxyl content was only 10%. This partially methylated polysaccharide was then subjected to three methylations by Kuhn's method (86) using dimethyl-formamide and methyl iodide at room temperature. The % OMe of the degraded polysaccharide was then found to be 21.4%. A further Kuhn methylation did not elevate the % OMe. The polysaccharide was therefore assumed to be fully methylated. Total yield of methylated degraded polysaccharide obtained was 1.14 g.

A portion of the methylated degraded gum (200 mg) was subjected to methanolysis with 3% methanolic hydrochloric acid (40 ml) for 5 hours. The acid solution was then neutralised with silver carbonate and the filtrate de-ionised with IR 120(H) resin. The neutral methanolic solution was then taken to dryness and the residue examined by gas chromatography and the methylated sugars found are shown in Table XIII.

TABLE XIII

Methyl glycosides of	T in system (b)	T in system (c)
2,3,5-tri-O-methyl-L-arabinose	0.54 and 0.71	0.43
2,5-di-O-methyl-L-arabinose	1.86 and 3.44	0.69
2,3,4-tri-O-methyl-L-arabinose	1.05	0.83
2,3,4,6-tetra-O-methyl-D-galactose	-	1.57 ?
2,4,6-tri-O-methyl-D-galactose	4.16 and 4.70	2.08 and 2.38
2,3,4-tri-O-methyl-D-galactose	7.48	2.93
2,4-di-O-methyl-D-galactose	-	4.06 and 4.75

The remainder of the methylated degraded polysaccharide C (600 mg) was subjected to methanolysis followed by hydrolysis with N-sulphuric acid, firstly at room temperature for 24 hours and then at 95° for 6 hours. The solution was neutralised with barium hydroxide and barium carbonate, filtered and finally de-ionised with Amberlite resin IR 120(H). Chromatographic examination of the syrup in solvents A and E revealed the presence of at least five di-, tri- or tetra- methylated sugars with no indication of any acidic or mono-methylated sugars.

The methylated sugars (460 mg) were separated by chromatography on a cellulose column (30 x 2.5 cm). The column was successively eluted with the following solvents:

Light petroleum (b.p. -100-120°) : butan-1-ol (7:3, saturated with water)

" " (1:1 ")

butan-1-ol, half-saturated with water and finally with water.

Fractions (7 ml) were collected every 15 min and every fourth fraction was taken to dryness and examined chromatographically in solvent E. In this way five final fractions were obtained, as shown in Table XIV.

TABLE XIV

Fraction	R_G	Colour	Wt. in mg.	Contents
1	0.96	black	41	2,3,5-tri-O-methyl-L-arabinose
	0.91	red		2,3,4,6-tetra-O-methyl-D-galactose
2	0.91	red	42	2,3,4,6-tetra-O-methyl-D-galactose
	0.86	black		2,5-di-O-methyl-L-arabinose
	0.84	pink	(trace)	2,3,4-tri-O-methyl-L-arabinose
3	0.82	red	164	2,4,6-tri-O-methyl-D-galactose
	0.78	reddish-brown		2,3,4-tri-O-methyl-D-galactose
4	0.60	reddish-brown	206	2,4-di-O-methyl-D-galactose
5			(trace)	monosaccharides

Fractions 1, 2 and 4 contained methylated sugars which were identical chromatographically to the methylated sugars previously obtained from methylation studies on the whole gum and whose identity was clearly established. These fractions, therefore, were only further separated on 3 MM paper and the amounts of individual methylated sugars established as below:

	Wt. in mg.
2,3,5-tri-O-methyl-L-arabinose	20
2,3,4,6-tetra-O-methyl-D-galactose	36
2,5-di-O-methyl-L-arabinose	35
2,3,4-tri-O-methyl-L-arabinose	trace
2,4-di-O-methyl-D-galactose	206

Fraction 3 was separated on 3 MM paper, two subfractions being obtained.

Subfraction 3a $R_G = 0.78$ $[\alpha]_D^{20}$ + 115° (C = 1.0 in water)

This fraction (142 mg) was chromatographically pure and identical to 2,3,4-tri-O-methyl-D-galactose in solvents A, E and G. Preparation of the aniline derivative gave a compound with m.p. 167°-168° which when admixed with an authentic sample of 2,3,4-tri-O-methyl-D-galactosylamine (m.p. 168°-170°) showed no depression in melting point.

Subfraction 3b R_G 0.82

This fraction (20 mg) was chromatographically identical to 2,4,6-tri-O-methyl-D-galactose.

DISCUSSION

To date gums from only a few of the many species of the genus Acacia have been investigated; of these, gum arabic (Acacia senegal syn verek) has been studied most extensively. The gums from Acacia mollissima, pycnantha cyanophylla, sundra and catechu have also been studied although not in such detail. Nevertheless, they appear to be of similar type, containing the same four sugars, namely, D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid.

The gum of Acacia seyal, which is the subject of the present work, had not been investigated previously although it is second only to Acacia senegal in commercial importance. It was thought of interest, therefore, to determine its relationship to other gums of the Acacia family.

Preliminary analytical studies on Acacia seyal (p.34) showed that not only was the gum heterogeneous (fractionation was effected on DEAE-cellulose) but that there were also variations in the proportion of sugars present in authenticated nodules collected from different trees. For structural studies the sample of Acacia seyal selected was nodule IX and the main methods of investigation were partial acid hydrolysis, methylation and periodate oxidation followed by a "Smith degradation".

A summary of the work carried out on Acacia seyal is given on page 92.

ACACIA SEYAL (DEL)

Comparative Analytical Studies on individual nodules

(a) Purification studies

(i) Precipitation

(ii) Ion-exchange

(iii) Electrodialysis

(b) Total Hydrolysis and molar proportions of sugars

(c) Viscosity measurements

(d) Equivalent weight determinations

(e) Heterogeneity studies

(f) Autohydrolysis

Structural Studies on nodule IX

(a) Graded Hydrolysis

(b) Partial Hydrolysis:

6-O-β-D-glucuronosyl-D-galactose

3-O-α-D-galactosyl-L-arabinose

3-O-β-L-arabinopyranosyl-L-arabinose

6-O-β-D-galactosyl-D-galactose

3-O-β-D-galactosyl-D-galactose

(c) Methylation of Gum:

2,3,4-tri-O-methyl-D-glucuronic acid

2,3,4-tri-O-methyl-L-rhamnose

2,3,5-tri-O-methyl-L-arabinose

2,3,4-tri-O-methyl-L-arabinose

2,5-di-O-methyl-L-arabinose

2,3-di-O-methyl-L-arabinose

2,3,4,6-tetra-O-methyl-D-galactose

2,4,6-tri-O-methyl-D-galactose

2,4-di-O-methyl-D-galactose

2-mono-O-methyl-D-galactose

(d) Smith degradation

degraded polysaccharides → methylation studies

2,3,5-tri-O-methyl-arabinose
2,3,4-tri-O-methyl-arabinose
2,5-di-O-methyl-arabinose
2,3,4,6-tetra-O-methyl-galactose
2,4,6-tri-O-methyl-D-galactose
2,3,4-tri-O-methyl-D-galactose
2,4-di-O-methyl-D-galactose

glycerol → monosaccharides, oligosaccharides

Partial Acid Hydrolysis

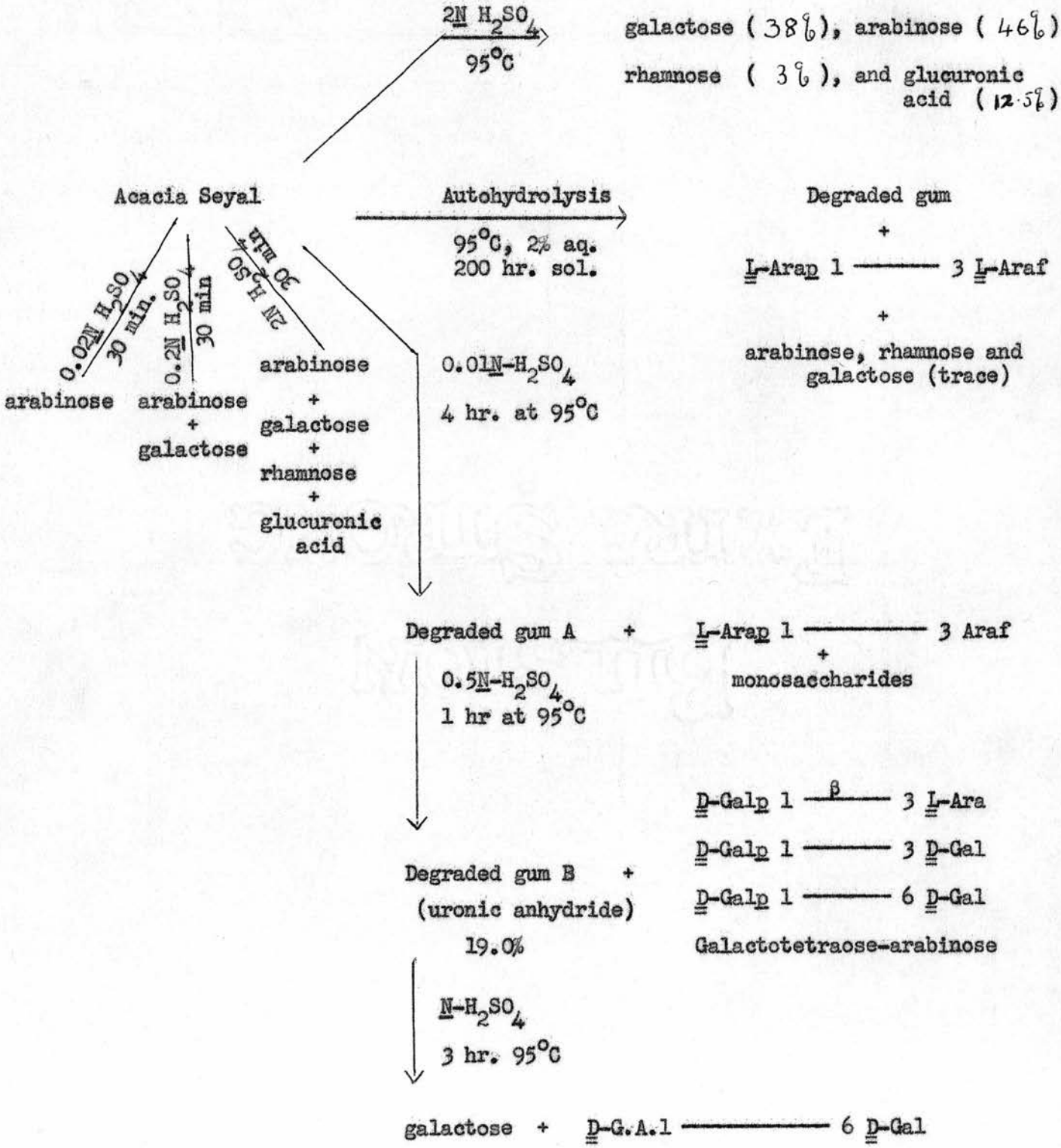
The molecular structure of plant gums usually involves different types of glycosidic linkages which have different degrees of stability towards acid hydrolysis.

In the present investigation partial hydrolyses were carried out with three different acid concentrations to yield fragments from the gum molecule as shown in the scheme on page 94.

Complete hydrolysis of the gum with $2\underline{N}$-H_2SO_4 (p.38) showed the presence of D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. In preliminary experiments involving partial acid hydrolysis of the gum under varying conditions, several oligosaccharides were detected as products. It was also noticed that some of the arabinose residues were removed under very mild acid conditions. The rhamnose residues were less labile and only very small amounts of galactose units were released.

Accordingly, graded acid hydrolysis was carried out in three stages as shown in the diagram, degraded gums A and B being separated from the soluble sugars after the first and second stages. At each stage the various oligosaccharides and monosaccharides were fractionated by adsorption and/or partition chromatography.

Scheme Showing Graded Hydrolysis



Mild acid hydrolysis resulted in the cleavage of most of the arabinose and rhamnose residues, leaving degraded gum A with a relatively resistant "core", composed largely of galactose, glucuronic acid and some arabinose and rhamnose residues. The disaccharide isolated at this stage appeared to be chromatographically homogeneous and gave only arabinose on hydrolysis. Methylation followed by hydrolysis of the disaccharide gave 2,4-di-O-methyl-L-arabinose and 2,3,4-tri-O-methyl-L-arabinose as the principal products with a small amount of 2,5-di-O-methyl-L-arabinose. These were identified by gas chromatography as well as by paper chromatography. Physical evidence (optical rotation + 201°) indicated that the disaccharide was

3-O-β-L-arabinopyranosyl-L-arabinose

The 2,5- and 2,4-methyl sugars would both be expected from the reducing residue of a 3'-linked arabinobiose, since in solution the pyranose- and furanose-rings exist together in equilibrium. This disaccharide has been isolated from several other Acacia gums (see p. 25).

Hydrolysis of the degraded gum A with 0.5N sulphuric acid gave the degraded gum B and a mixture of oligosaccharides and monosaccharides. The oligosaccharides were purified by fractionation on a charcoal-celite column followed by partition chromatography on cellulose. The main products at this stage were galactose-containing oligosaccharides together with 3-O-β-L-arabinopyranosyl-L-arabinose.

The first fraction contained a disaccharide which, on complete methylation followed by hydrolysis, gave equimolecular proportions of 2,3,4,6-tetra- and 2,3,4-tri-O-methyl-D-galactose, proving the structure of the disaccharide to be 6-O-β-D-galactopyranosyl-D-galactose. The

presence of a β-linkage was indicated by the low positive rotation ($[\alpha]_D$ + 33°) of the disaccharide, which was similar to that recorded for a 1,6-linked galactobiose isolated from a partial acid hydrolysate of gum ghatti (103).

The second fraction obtained contained two disaccharides both containing arabinose. The first member gave on hydrolysis approximately equimolecular amounts of galactose and arabinose and on methylation followed by hydrolysis the main methylated sugars identified by crystalline derivatives were 2,3,4,6-tetra-O-methyl-D-galactose and 2,4-di-O-methyl-L-arabinose.

This disaccharide also had $[\alpha]_D$ + 156° and on the strength of the above evidence it was thought to be

3-O-α-D-galactopyranosyl-L-arabinose

Similar results were obtained by Charlson et al. (73) from Acacia cyanophylla gum.

The second member of this fraction was identified as above to be

3-O-β-L-arabinopyranosyl-L-arabinose.

The final oligosaccharide fully characterised at this stage of acid hydrolysis was the 1-3-linked galactobiose

3-O-β-D-galactopyranosyl-D-galactose

This disaccharide on methylation and hydrolysis furnished 2,3,4,6-tetra- and 2,4,6-tri-O-methyl-D-galactose as main products, indicating a 1-3-linkage. It was also obtained in the crystalline form and comparison of the physical constants with that of an authentic sample of 3-O-β-D-galactopyranosyl-D-galactose obtained from Anogeissus schimperi gum proved its identity.

In addition to the above oligosaccharides a polygalactose-arabinose oligosaccharide was obtained in very small quantity which prevented further investigation other than optical activity ($[\alpha]_D$ + 18 ± 2°) and total hydrolysis followed by estimation of relative amounts of galactose to arabinose. This was found to be 10 : 1. On account of its optical activity this oligosaccharide could be compared to a tetragalactose-arabinose oligosaccharide which was isolated from Combretum leonense gum (21) and a similar one isolated from gum ghatti (103).

The only aldobiouronic acid isolated and characterised was

6-O-β-D-glucuronopyranosyl-D-galactose

It was obtained from the third stage of partial acid hydrolysis, namely, hydrolysis of degraded gum B with N-sulphuric acid. Methylation of this aldobiouronic acid, followed by methanolysis, borohydride reduction and hydrolysis yielded glucose and galactose. Chromatographically this aldobiouronic acid was homogeneous and identical to an authentic sample of 6-O-β-D-glucuronopyranosyl-D-galactose isolated from gum ghatti (103).

Methylation and Smith Degradation Studies on Gum

The undegraded gum, purified by electrodialysis, was methylated with methyl sulphate and sodium hydroxide; further treatment of a portion with methyl iodide and silver oxide had no significant effect on the methoxyl content. The methylated polysaccharide (OMe, 38.5%) was precipitated from petroleum ether (b.p. 60-80°) and then hydrolysed with sulphuric acid. A good separation of the neutral and acidic (as barium salts) methylated sugars was achieved by preliminary fractionation on a cellulose column.

The mixture of neutral sugars was fractionated by partition chromatography on cellulose and the following sugars were characterised by formation of crystalline derivatives and/or by crystallisation of the sugars as well as by gas chromatography of their methyl glycosides:

	Approx. weight
2,3,5-tri-O-methyl-L-arabinose	320 mg
2,5-di-O-methyl-L-arabinose	395 mg
2,3-di-O-methyl-L-arabinose	35 mg
2,3,4-tri-O-methyl-L-arabinose	85 mg
2,3,4,6-tetra-O-methyl-D-galactose	190 mg
2,4,6-tri-O-methyl-D-galactose	265 mg
2,4-di-O-methyl-D-galactose	470 mg
2,3,4-tri-O-methyl-L-rhamnose	100 mg

In addition to the above major components small amounts of 2-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-galactose were also detected (by paper chromatography) together with some arabinose, galactose and rhamnose.

The acidic fraction which was only slightly contaminated by some monosaccharides and a dimethylated sugar, was, after separation from

the latter on 3 MM paper, converted into the corresponding mixture of methyl ester methyl glycosides, reduced with lithium aluminium hydride and hydrolysed to give a mixture of two neutral sugars, identified as 2,3,4-tri-O-methyl-D-glucose and glucose itself.

Methylation of degraded gum B (obtained after second stage of partial acid hydrolysis) was carried out with methyl iodide and barium oxide. Owing to the very small amount of gum available for methylation (1 g) the products after hydrolysis could only be detected by paper chromatography as following:

2,3,4,6-tetra-O-methyl-D-galactose (trace)

2,4,6-tri-O-methyl-D-galactose

2,3,4-tri-O-methyl-D-galactose (heavy spot)

2,4-di-O-methyl-D-galactose (heavy spot)

2,3,4-tri-O-methyl-D-glucuronic acid (heavy spot)

with no arabinose or rhamnose derivatives detected. This was in agreement with the hydrolysis results which gave only galactose and glucuronic acid (19%) as products.

The whole gum was finally subjected to periodate oxidation, followed by Smith degradation. The gum (14 g) was dissolved in water and oxidised with sodium periodate over a period of 36 hours at room temperature in the absence of light. Excess periodate was destroyed by ethylene glycol and the solution was dialysed to remove small molecular weight materials. The solution was then concentrated to half-volume and reduction was effected with potassium borohydride. After two days the reaction was stopped, excess borohydride was destroyed by addition of Amberlite resin IR 120(H). Further treatment with some resin and several evaporations with methanol, effected removal of inorganic ions. The polyalcohol thus

obtained was dissolved in N-sulphuric acid at room temperature and left for three hours. Neutralisation was effected with barium hydroxide and barium carbonate followed by removal of ions with Amberlite resin IR 120(H). After further concentration of solution, the degraded polysaccharide was precipitated by addition of four volumes of ethanol. It was removed on the centrifuge and dried and the supernatant solution was concentrated and examined by paper chromatography. The latter proved to consist predominantly of glycerol (85-90%) together with some arabinose and rhamnose.

The degraded gum (6 g) was dissolved in water and six methylations were carried out with dimethyl sulphate and sodium hydroxide, followed by four methylations by Kuhn's method (86). The methoxyl content was observed to be 21.4%.

The methylated material (1.14 g) was methanolysed and a portion of the syrup was examined by gas chromatography when the following sugars were detected:

Methyl glycosides of	T in system (b)	T in system (c)
2,3,5-tri-O-methyl-L-arabinose	0.54 and 0.71	0.43
2,5-di-O-methyl-L-arabinose	1.86 and 3.44	0.69
2,3,4-tri-O-methyl-L-arabinose	1.05	0.83
2,3,4,6-tetra-O-methyl-D-galactose	-	1.57 ?
2,4,6-tri-O-methyl-D-galactose	4.16 and 4.70	2.08 and 2.38
2,3,4-tri-O-methyl-D-galactose	7.48	2.93
2,4-di-O-methyl-D-galactose	-	4.06 and 4.75

The remainder of the syrup was hydrolysed with N-sulphuric acid, first at room temperature for 24 hours and finally at 95°C for 4 hours. After neutralisation, paper chromatography showed the presence of at least five

methylated sugars which were, in the first place, separated on a cellulose column and then on thick paper giving the following:

	Approx. weight
2,3,5-tri-O-methyl-L-arabinose	20 mg
2,5-di-O-methyl-L-arabinose	35 mg
2,3,4-tri-O-methyl-L-arabinose	trace
2,3,4,6-tetra-O-methyl-D-galactose	36 mg
2,4,6-tri-O-methyl-D-galactose	20 mg
2,3,4-tri-O-methyl-D-galactose	144 mg
2,4-di-O-methyl-D-galactose	206 mg

The evidence for their identities was obtained by paper chromatography (except for 2,3,4-tri-O-methyl-D-galactose, which was identified by preparation of crystalline aniline derivative), comparison being made with methylated sugars previously fully characterised in solvent systems A, E and G.

Hydrolysis products of methylated Acacia seyal gum and of Smith degraded methylated Acacia seyal gum.

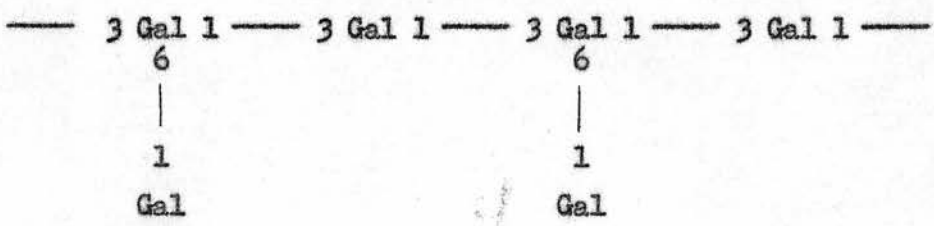
	Relative Amounts	
	Undegraded	Degraded
2,3,4,6-tetra-O-methyl-D-galactose	++	+
2,4,6-tri-O-methyl-D-galactose	++	+
2,3,4-tri-O-methyl-D-galactose	trace	++
2,4-di-O-methyl-D-galactose	++++	++++
2-mono-O-methyl-D-galactose	trace	-
3-mono-O-methyl-D-galactose	trace	-
2,3,5-tri-O-methyl-L-arabinose	+++	+
2,3,4-tri-O-methyl-L-arabinose	+	trace
2,5-di-O-methyl-L-arabinose	+++	+
2,3-di-O-methyl-L-arabinose	trace	-
2,3,4-tri-O-methyl-D-glucuronic acid	++	-
2,3,4-tri-O-methyl-D-rhamnose	+	-

Structural features of the gum

The structural features of Acacia seyal gum, on the basis of the foregoing results, can be conveniently discussed by considering, in turn, the galactan framework and the acid-labile units.

The Galactan Framework

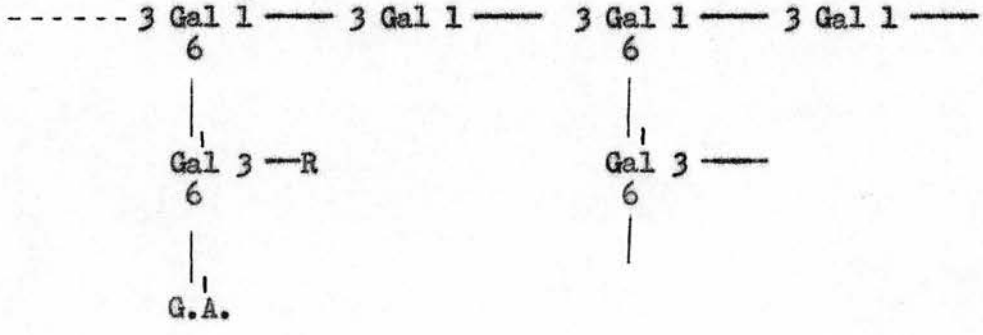
Since very little galactose and no galactose-containing oligosaccharides were among the low molecular weight materials of mild acid hydrolysis of the gum, it may be assumed that the framework of the gum is essentially made up of galactose residues. The isolation of 1,3- and 1,6-linked galactobioses from the partial acid hydrolysate suggests that the galactan framework is made up of a highly branched system of 1,3- and 1,6-linked galactopyranose units. Since,however, the proportion of 1,3- to 1,6-linked units isolated was approximately in the ratio of 2 : 1, it would appear that the main chain is made up of 1,3-linked galactose units and that the 1,6-linkages are those of galactose residues attached as side-chains.



This view is supported by the fact that the two most plentiful products of methylation are 2,4,6-tri-O-methyl-D-galactose and 2,4-di-O-methyl-D-galactose. Similarly, oxidation of the gum resulted in the consumption of 0.71 mole of periodate per sugar unit. Many of the sugar residues in the gum were not attacked by the periodate at all and this could also mean that the polysaccharide contains a fair proportion of 1,3-glycosidic

linkages. Further, the large proportion of 2,3,4-tri-O-methyl-D-galactose isolated from the methylated Smith degraded polysaccharide, as well as its presence in the methylated acid-degraded gum, seems to indicate that the side-chains which are either acid-labile or open to oxidation are attached to carbon atom 3 in the original gum.

Assuming that the glucuronic acid residues are present in the pyranose form, the isolation of 2,3,4-tri-O-methyl-D-glucuronic acid together with the aldobiouronic acid 1,6-glucuronopyranosyl-D-galactose from partial acid hydrolysis indicates that the uronic acid is linked through position 1 to a galactose unit in position 6 and must be an end group. The absence of 2,3-di-O-methyl-D-glucuronic acid seems to point to the absence of 1,4-linkages, found in Acacia senegal (74, 57). There is no evidence, as yet, however, to decide whether the aldobiouronic acid acid groups are attached directly to the main chain or whether one or more galactose units are interposed. The latter view is the most probable one, however, taking into account the large proportion of 2,4-di-O-methyl-D-galactose obtained not only from the methylated whole gum but also from the degraded gums.



Acid Labile Residues

As in the case of the other Acacia gums so far investigated, mild acid hydrolysis removes a large proportion of the arabinose content together with most of the rhamnose. It is therefore probable that these are in the outer parts of the molecule, and that many of the arabinose residues are in the furanose form. This has been confirmed by methylation studies on the whole gum which led to the isolation of a large proportion of 2,3,5-tri-O-methyl-L-arabinose. The characterisation of 3-O-β-L-arabinopyranosyl-L-arabinose as a partial acid hydrolysis product of the whole gum establishes the presence of adjacent L-arabinose residues in the gum, as well as the fact that some of the L-arabinose end groups are in the pyranose form. This was further confirmed by the presence of 2,3,4-tri-O-methyl-L-arabinose in the hydrolysis products of the methylated gum. Although no other arabinobioses have been characterised as partial acid hydrolysis products, it is probable that the outer chains contain some other adjacent arabinose residues since mild acid hydrolysis of the gum removes some of the non-terminal L-arabinofuranose units as proved by the isolation of 3-O-α-D-galactopyranosyl-L-arabinose. Furthermore, reduction of the periodate oxidised gum, followed by mild hydrolysis, affords a degraded gum still containing arabinofuranose end groups; it is probable that these units originated from non-terminal arabinose residues in the outer chains, which are also resistant to attack by periodate, since the degradation procedure would remove the outer shell of arabinofuranose end-groups. Similarly the presence of 2,3,4-tri-O-methyl-L-arabinose could indicate an arabinose unit which occurred in the undegraded gum as a 3-substituted arabinopyranose residue. However, the very small amount of this sugar present together with the absence of the 2,4-methyl derivative, throws some doubt on this observation.

Even though the rhamnose and some of the galactose residues were relatively easily removed on mild acid hydrolysis, subsequent methylation studies showed that they were in the pyranose form. In this connection it may be noted that Bouveng (104) failed to find any significant difference in the rates at which the galactopyranosyl residues and arabinofuranosyl residues were released from the arabinogalactan of Western Larch on mild hydrolysis. In view of the amounts of 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-rhamnose isolated, these must also form terminal groups in the gum; further, an appreciable proportion of non-reducing terminal galactose units must be present in the Smith degraded polysaccharide owing to a finite, although small, amount of 2,3,4,6-tetra-O-methyl-D-galactose found. Thus, taking into account the above results, the main acid-labile groups in the gum are:

Araf 1 ———

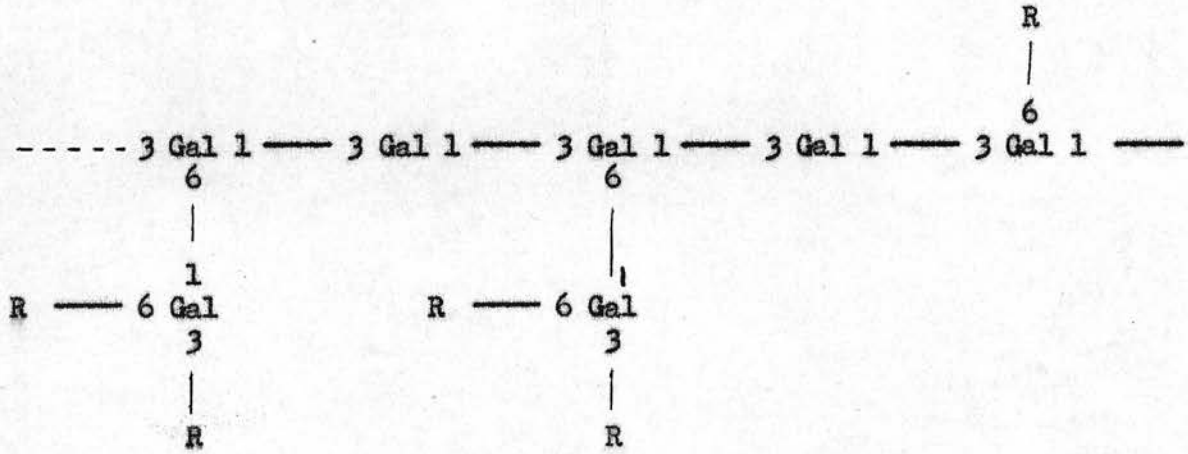
Rhp 1 ———

Arap 1 —β— 3 Araf

Galp 1 —α— 3 Araf

Galp 1 ———

Thus most of the main structural features of *Acacia seyal* are now known and although it is not possible to put forward a complete structure, the following general representation is in agreement with most of the experimental evidence:



R = Araf 1 .....

or Rhp 1 .....

or Galp 1 —— 3 Araf .....

or Arap 1 —— 3 Araf .....

or G.A. 1 .....

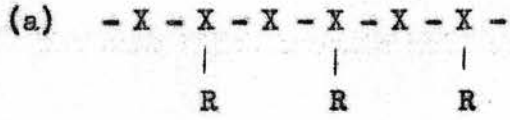
Comparison of the structural features of Acacia seyal with those of Acacia senegal and Acacia pycnantha.

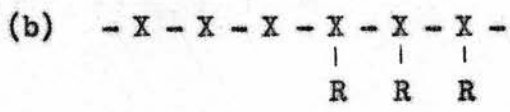
As a conclusion, the main structural features of Acacia seyal will be compared with those of two other Acacia gums, namely, Acacia senegal and Acacia pycnantha.

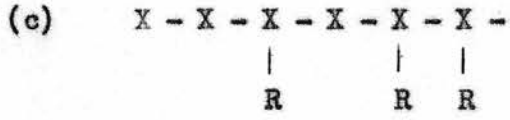
The three gums show similarities in their basic structures and contain the same four component sugars (D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid), in different relative amounts. Acacia seyal contains more L-arabinose than D-galactose whilst Acacia pycnantha and Acacia senegal contain more D-galactose than L-arabinose. Variations in L-rhamnose and D-glucuronic acid content also occur.

All three gums contain highly branched frameworks of galactose residues in which the main chain is linked 1 - 3, and the side chains 1 - 6. However, in the more complex Acacia senegal, each galactose unit in the backbone carries a galactose containing side-chain, whereas, in Acacia pycnantha and Acacia seyal such side-chains are attached only to approximately every second main chain galactose unit.

In such circumstances, the attachment of the side-chains may occur (a) in a regular alternating pattern (b) in a regular recurring block pattern (c) in completely random fashion. These three possibilities are indicated in the diagrams below. The present evidence is unable to make any distinction between these.

(a)


(b)


(c)


X = main-chain D-galactose units, linked β 1 - 3.

R = side-chains, attached to C6 of D-galactose residues.

It would, therefore, be interesting to apply the elegant degradative procedure developed by Smith et al. (70) to the acid "degraded" gum to try and establish the distribution of the branched points along the chain as has been done by Aspinall (105) on a rye flour arabinoxylan where the distribution of arabinofuranose side-chains was found to be quite random.

The same aldobiouronic acid occurs in the side chains of all three gums and indeed the acid has been isolated from all the Acacia gums so far investigated. In Acacia pycnantha and Acacia seyal gums the acid disaccharide grouping occurs exclusively as end groups, but there is evidence (57) that it exists in Acacia senegal with attached acid labile groupings as well as being in the terminal position.

The same monosaccharides, L-arabinose and L-rhamnose are liberated on mild acid hydrolysis of the three gums but the more complex acid labile groupings differ. In Acacia seyal and Acacia senegal two

such groupings have been isolated namely 3-O-β-L-arabinopyranosyl-L-arabinose and 3-O-α-D-galactosyl-L-arabinose, whilst in Acacia pycnantha only 3-O-β-L-arabinofuranosyl-L-arabinose has been isolated.

Finally the positions to which the specific acid-labile groups are attached are still unknown but the points of linkage are known and these are different. In Acacia senegal the groupings are joined to C atom 3 of galactose residues in the outer chains and to C atom 4 of the glucuronic acid units, whereas, in Acacia pycnantha gum, they are linked to C atom 6 of the galactose residues in both "core" and side-chains. In Acacia seyal they appear to be joined to both C atoms 3 and 6 of the galactose units.

Thus Acacia seyal seems to incorporate a few of the structural features from both of the other two Acacia gums and in many ways seems to be intermediate to them.

ACKNOWLEDGEMENTS

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My sincere thanks are also due to Dr. D.M.W. Anderson for his expert guidance and friendly encouragement.

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Marek A. Herbich

BIBLIOGRAPHY

1. H.S. Blunt, "Gum Arabic, with Special Reference to its Production in the Sudan", Oxford Univ. Press, London and New York, 1926.
2. D.W. Malcolm, "Report on Gum and Gum Arabic", Govt. Printer, Dar-es-Salaam, 1936.
3. J.K. Hamilton, D.R. Spriestersbach and F. Smith, J. Amer. Chem. Soc., 1957, 79, 443.
4. E. Anderson and B.B. Blake, J. Amer. Pharm. Assc., 1953, 42, 662.
5. C. Neubauer, J. Prakt. Chem., 1854, 62, 193.
6. C. Scheibler, Ber., 1873, 6, 612.
7. C. O'Sullivan, J. Chem. Soc., 1884, 41.
8. R.L. Whistler, "Industrial Gums", Academic Press, New York, 1959.
9. B.A. Lewis and F. Smith, J. Amer. Chem. Soc., 1957, 79, 3929.
10. F.J. Joubert, J. S. African Chem. Inst., 1954, 107.
11. J.D. Geerdes, B.A. Lewis, and F. Smith, J. Amer. Chem. Soc., 1957, 79, 4209.
12. L. Adriaens, Chem. Abstracts, 1943, 37, 4926.
13. R. Taft and L.E. Malm, J. Phys. Chem., 1931, 35, 874.
14. S.N. Banerji, J. Indian Chem. Soc., 1952, 29, 270.
15. D.R. Briggs and M. Manig, J. Phys. Chem., 1944, 48, 1.
16. F. Smith and R. Montgomery, "The Chemistry of Plant Gums and Mucilages", Reinhold Publishing Corporation, New York, 1959.
17. M. Heidelberger and J. Adams, J. Exptl. Med., 1956, 103, 189.
18. H. Neukom, H. Deuel, W.J. Heri, and W. Kundig, Helv. Chem. Acta, 1960, 43, 64.
19. D.M.W. Anderson, E.L. Hirst and N. King, Talanta, 1959, 3, 118.
20. E.L. Hirst, J. Chem. Soc., 1955, 2974.
21. V. Bhavanandam, Ph.D. Thesis, University of Edinburgh, 1962.
22. D.M.W. Anderson and J.F. Smith, private communication.

23. G.O. Aspinall, E.L. Hirst, and N.K. Matheson, J. Chem. Soc., 1956, 989.

24. T. Christensen, Ph.D. Thesis, University of Edinburgh, 1959.

25. D.M.W. Anderson, and N.J. King, Talanta, 1961, 8, 497.

26. D.W. Drummond and E.E. Percival, J. Chem. Soc., 1961, 3908.

27. S.P. James and F. Smith, J. Chem. Soc., 1945, 739, and 749.

28. E. Hoagland, B. Lindberg and J. McPherson, Acta Chem. Scand., 1956, 10, 1160.

29. H.E. Khaden and M.M. Megahed, J. Chem. Soc., 1956, 3953.

30. G.O. Stephen, M.J. Johnstone and A.M. Stephen, J. Chem. Soc., 1960, 4918.

31. F. Smith and H.C. Srivastava, J. Amer. Chem. Soc., 1959, 81, 1715.

32. D.G. Easterby and J.K.N. Jones, Nature, 1950, 165.

33. E.L. Hirst and S.J. Dunstan, J. Chem. Soc., 1953, 2332.

34. B.C. Bera, A.B. Foster and M. Stacey, J. Chem. Soc., 1955, 3788.

35. S.A. Barker, M. Stacey, and G. Zweifel, Chem. and Ind., 1957, 330.

36. S. Gardell, Acta Chem. Scand., 1957, 11, 668.

37. A.E. Goodban and H.S. Owens, J. Polymer Sci., 1957, 23, 825.

38. B. Siegel, G.A. Candela and R.M. Howard, J. Amer. Chem. Soc., 1954, 76, 1311.

39. W. Heri, H. Neukom and H. Deuel, Helv. Chim. Acta, 1961, 44, 1939.

40. K.C.B. Wilkie, J.K.N. Jones, B.J. Excell and R.E. Semple, Canad. J. Chem., 1957, 35, 795.

41. H. Spitzy, H. Skrube and K. Müller, Mikrochim Acta, 1961, 296.

42. R.L. Kislink, Biochem. biophys. Acta, 1960, 531.

43. G. Ostling, Acta Soc. Med. Upsaliensis, 65, 1960, 222.

44. C.G. Belling, Nature, 1961, 192, 326.

45. A. Bill, N. Marsden, and H.R. Ulfendahl, Scand. J. Clin. Lab. Invest., 12, 1960, 392.

46. C.T. Greenwood, "Advances in Carbohydrate Chemistry", 1952, 7, 289.

47. W.N. Haworth, J. Chem. Soc., 1915, 107, 8.

48. T. Purdie and J.C. Irvine, J. Chem. Soc., 1903, 1021.

49. R. Kuhn, H. Trischmann and I. Löw, Angen. Chemie, 1955, 67, 32.

50. C.M. Fear and R.C. Menzies, J. Chem. Soc., 1926, 937.

51. L. Hough and J.K.N. Jones, Chem. and Ind. 1952, 30, 380.

52. I. Mukat, J. Amer. Chem. Soc., 1934, 54, 2449.

53. H.S. Isbell, H.L. Frush, B.H. Bruchner, G.N. Kowkabany and G. Wampler, Analyt. Chem., 1957, 29, 1523.

54. C.T. Bishop and F.P. Cooper, Canad. J. Chem., 1960, 38, 388.

55. G.O. Aspinall, J. Chem. Soc., 1963, 1676.

56. J.K.N. Jones and W.H. Nicholson, J. Chem. Soc., 1958, 27.

57. G.O. Aspinall, A.J. Charbon, E.L. Hirst and R. Young, J. Chem. Soc., 1963, 1696.

58. G.O. Aspinall, I.M. Cairncross and A. Nicolson, Proc. of Chem. Soc., 1959, 270.

59. R.L. Whistler and D.F. Durso, J. Amer. Chem. Soc., 1950, 72, 677.

60. L. Hough, J.K.N. Jones, W.H. Wadman, J. Chem. Soc., 1949, 2511.

61. J.K.N. Jones, R.A. Wall, and A.O. Pittet, Canad. J. Chem., 1960, 38, 2285.

62. J.M. Bobbit, "Advances in Carbohydrate Chemistry", 1956, 11, 1.

63. A.S. Perlin, "Advances in Carbohydrate Chemistry", 1959, 14, 9.

64. L. Malaprade, Bull. Soc. Chim., 1928, 43, 683.

65. H. Klostermann and F. Smith, J. Amer. Chem. Soc., 1952, 74, 5336.

66. V.C. Barry and P.W.D. Mitchell, J. Chem. Soc., 1953, 3631.

67. A. Jeans and J.C. Rankin, J. Amer. Chem. Soc., 1954, 74, 4438.

68. P.F. Fleury and J. Lange, J. Pharm. Chim., 1933, 17, 107.

69. G.O. Aspinall and R.J. Ferrier, Chem. and Ind., 1957, 2271.

70. I.J. Goldstein, G.W. Hay, B.A. Lewis and F. Smith, Abstracts, 135th A.C.S. Meeting, Boston, Mass. 1959.

71. V.C. Barry, Nature, 1943, 152, 537.

72. A.J. Charbon, J.R. Nunn, and A.M. Stephen, J. Chem. Soc., 1955, 1428.

73. A.J. Charbon, J.R. Nunn, and A.M.Stephen, J. Chem. Soc., 1955, 269.

74. F. Smith, J. Chem. Soc., 1940, 79.

75. A.M. Stephen, J. Chem. Soc., 1951, 646.

76. R.K. Hulyalkar, T.R. Ingle, and B.V. Bhide, J. Indian Chem. Soc., 1956, 33, 861

77. E.L. Hirst and A.S. Perlin, J. Chem. Soc., 1954, 2622.

78. F. Smith, J. Chem. Soc., 1940, 1035.

79. S. Mukherjee and A.N. Shrivastava, J. Amer. Chem. Soc., 1958, 80, 2536.

80. F. Smith, J. Chem. Soc., 1939, 744.

81. F. Smith and D. Spriestersbach, Abstracts, 1955, A.C.S. Meeting, Minneapolis.

82. G.O. Aspinall, E.L. Hirst and A. Nicolson, J. Chem. Soc., 1959, 1697.

83. D.M.W. Anderson, Talanta, 1959, 2, 73.

84. D.M.W. Anderson, and J.L. Duncan, Talanta, 1961, 8, 241.

85. J.A. Cinofelli and F. Smith, Analyt. Chem., 1954, 26, 1132.

86. R. Kuhn, E. Egge, R. Brossmer, A. Gauke, P. Klesse, W. Lochinger, E. Röhm, H. Trischmann, and D. Tschampel, Angen. Chem., 1960, 72, 805.

87. T.G. Bonner, E.J. Bourne and S. McNally, J. Chem. Soc., 1960, 2929.

88. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, Analyt. Chem., 1956, 28, 350.

89. D.M.W. Anderson, S. Garbutt and S.S.H. Zaidi, Analyt. Chim. Acta, in press.

90. M. Heidelberger, J. Adams and Z. Dische, J. Amer. Chem. Soc., 1956, 78, 2853.

91. C.L. Butler and L.H. Critcher, J. Amer. Chem. Soc., 1931, 53, 4160.

92. E.L. Hirst and J.K.N. Jones, J. Chem. Soc., 1938, 1174.

93. C.T. Greenwood and N.K. Mathieson, Chem. and Ind., 1956, 988.

94. A.W. Thomas and H.A. Murray, J. Phys. Chem., 1928, 32, 676.

95. A.M. Anderson and C.B. Wylam, Chem. and Ind., 1956, 191.

96. C.T. Greenwood and J. Thomson, J. Chem. Soc., 1962, 222.

97. Personal communication, Vidal-Hall, Gum Research Officer, Sudan.

98. M. Stacey, Chem. and Ind., 1943, 62, 110.

99. J.K.N. Jones and F. Smith, "Advances in Carbohydrate Chemistry", 4, 243.

100. J.C. Perrone, L.V. Disitzer, and G. Dormont, Nature, 1959, 183, 605.

101. R.J. Colvin, W.H. Cook, and G.A. Adams, Canad. J. Chem., 1952, 30, 603.

102. M. Somogyi, J. Biol. Chem., 1952, 195, 19.

103. B.A. Auret, Ph.D. Thesis, University of Edinburgh, 1958.

104. H.O. Bouveng, Acta Chem. Scand., 1961, 15, 78.

105. G.O. Aspinall and K.M. Ross, J. Chem. Soc., 1963, 1681.

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1. *Studies on Uronic Acid Materials. Part VI.* The Variation in Composition and Properties of Gum Nodules from* Acacia seyal *Del.*

By D. M. W. Anderson and M. A. Herbich.

As a preliminary to studies of the chemical structure of *Acacia seyal* gum, individual nodules of authenticated origin have been examined as crude gum and after purification by (i) precipitation, (ii) electrodialysis, and (iii) ion-exchange. Chemical composition and physical behaviour vary from nodule to nodule. The low natural nitrogen content is not decreased by any of the purification methods used. Passage through a column of diethylaminoethylcellulose shows that the purified gum from individual nodules is heterogeneous, two components being present. The proportion of component A in different nodules varies from 34% to 41%; components A and B contain 12·5% and 15·3%, respectively, of glucuronic acid.

As a result of specific immunological reactions [1] and electrophoresis studies,[2,3] it is now accepted [4-6] that gum arabic (*Acacia senegal* syn. *verek*) is a mixture of polysaccharides of similar composition; [1] no single over-all formula has significance,[5] and only general features can be indicated.[4] Early studies [7] have been criticised [5,6,8] on the grounds that composite commercial samples, inadequately authenticated, were used. Although it had been suspected,[1,9] despite some evidence to the contrary,[10] that different samples of certain plant gums varied in chemical constitution, the possible range of variation was not known until single nodules of *Combretum leonense* gum were studied.[11] The results implied that fine-structural differences exist from nodule to nodule, so that alcoholic precipitation of bulk material from an aqueous solution of many nodules produces a complex mixture of closely similar polymeric systems. Whenever sample size permits, it is therefore desirable to assess the extent of inter-nodule variation and to make structural studies on the simplest form of the polymer available, *i.e.*, that given by a single nodule, which itself may be polymolecular and/or polydisperse (terminology as in ref. 12).

Before studying the chemical structure of the components of *A. seyal* gum, we have investigated the extent to which a number of authenticated nodules vary in properties, in composition, and in heterogeneity (cf. ref. 6, p. 54).

Experimental

Collection and Origin of Specimens.—We are grateful to Mr. P. Vidal-Hall, Gum Research Officer to the Sudan Government, who collected suitable gum nodules from the red-barked *A. seyal* Del. (a close variant, *A. seyal* var. *fistula*, has a grey bark). *A. seyal* is not normally "tapped," and the nodules originated from "natural exudation." The nodules, taken only from trees which could be authenticated, were packed individually and despatched in sealed tins. Nodules I—VI were collected at Umm Ruaba Forest Reserve, Eastern Kordofan, on March 9th, 1960; nodules VII and VIII from El Ain Forest Reserve, Central Kordofan (700 miles distant from Umm Ruaba), on January 9th, 1961. Sample IX was a representative bulk sample of first quality commercial "gum talh" (*A. seyal*). Nodules I—VIII ranged in weight from 40 to 80 g.; their colour varied from pale yellow to dark brown. Nodules I—IV, VII, and VIII were clear and glassy, of spherical shape. Nodules V and VI were elongated and had a characteristic glazed appearance, which, we have since observed, results when nodules plasticise slightly at 90—100°. It therefore appears that nodules V and VI had been subjected to more vigorous natural drying conditions than the others; it is unlikely that they were products of an earlier season, since *A. seyal* nodules (unlike *A. senegal*) become brittle through dehydration and fall from the branches within a few months.

* Part V, Anderson, Garbutt, and Smith, *Talanta*, 1962, **9**, 689.

Analytical Methods.—The standard methods,[11] were used, namely: paper partition chromatography; determination of sugars liberated on hydrolysis; autohydrolysis; electrophoresis; and viscosity experiments. The suspended-level dilution viscometer had a water flow-time of 218 sec. at 25°. Methoxyl contents were found by the vapour-phase infrared method,[13] which distinguishes yields of methyl iodide from other volatile products arising from solvent retention, decomposition, etc. Results were corrected for moisture content. Optical rotations were found for 1% aqueous solutions.

Studies on Crude Material.—The nodules, individually crushed to pass a 30-mesh sieve, gave the results shown in Table 1.

TABLE 1.

Determinations on crude samples.

	I	II	III	IV	V	VI	VII	VIII	IX
Moisture (%)	13·5	13·6	13·3	14·3	11·0	11·0	16·1	15·9	11·4
	13·6	13·6	13·2	14·4	11·0	11·2	16·0	16·0	11·4
Ash (%)	3·42	2·81	2·89	3·31	2·04	1·94	2·90	2·70	3·55
	3·38	2·98	2·94	3·33	2·10	2·10	2·94	2·80	3·61
Nitrogen (%)	0·14	0·09	0·14	0·12	0·09	0·10	0·18	0·17	0·19
	0·15	0·10	0·14	0·13	0·10	0·10	0·19	0·18	0·19
Uronic anhydride (%)	12·4	12·2	12·1	11·2	9·0	9·2	16·4	12·1	11·6
	12·7	12·4	12·0	11·4	9·1	9·2	16·8	11·9	11·9
Methoxyl (%)	n.d.	n.d.	0·60	0·72	1·36	1·53	1·00	0·82	0·55
Limiting flow-time no.	8·7	8·7	12·7	14·7	8·2	9·8	15·3	19·0	15·6
$[\alpha]_D^{20}$	+52°	n.d.	+56°	+51°	n.d.	+48°	n.d.	+50°	+44°

n.d. = Not determined.

Autohydrolysis at 85—90° of 1% solutions of nodules III, V, and VIII gave arabinose, together with traces of galactose and an oligosaccharide. As was observed for *C. leonense* gum,[11] the increase in reducing power (see Fig. 1) varies from nodule to nodule. The acidity of the autohydrolysis solutions (pH 4·6) did not increase appreciably with time of heating (cf. *C. leonense*,[11] which had an appreciable acetyl content) and decomposition of the liberated sugars was not extensive. The viscosity of the solutions fell rapidly during autohydrolysis.

Purification of Crude Gum.—A portion of each crushed nodule was shaken with cold distilled water, to give a 2% solution, which was filtered through acid-hardened filter-paper. The solutions were acidified (0·1N in hydrochloric acid); addition of acetone (4 volumes) gave a white curdy precipitate which was removed by centrifugation. Further precipitation did not occur when the clear supernatant liquid was poured into acetone. This purification process was carried out a further 3 times: the purified gum was then dialysed against distilled water and freeze-dried.

Studies on Samples Purified by Precipitation.—The results obtained are compared in Table 2. For the determinations of the limiting flow-time number, 4% saline was found to give adequate

TABLE 2.

Determinations on samples purified by precipitation.

	I	II	III	IV	V	VI	VII	VIII	IX
Ash (%)	2·38	1·81	2·71	2·49	0·91	n.d.	n.d.	n.d.	2·48
	2·41	1·82	2·78	2·51	0·92				2·52
Nitrogen (%)	0·14	0·09	0·13	0·16	0·07	0·10	0·18	0·17	0·20
	0·15	0·10	0·13	0·17	0·07	0·10	0·19	0·18	0·21
Uronic anhydride (%)	13·1	12·8	12·5	12·8	10·4	10·9	16·6	12·9	12·4
	12·9	12·9	12·7	12·6	10·6	10·8	16·4	12·7	12·6
Methoxyl (%)	n.d.	1·1	1·3	0·70	1·1	n.d.	n.d.	1·0	0·94
Limiting flow-time no.	11·4	9·1	12·0	12·8	7·4	11·6	13·8	17·4	13·2
$[\alpha]_D^{20}$	+58°	n.d.	+59°	n.d.	n.d.	n.d.	n.d.	+64°	n.d.

suppression of the electroviscous effect. Although the uronic anhydride content of each sample was greater than that in the crude gum, indicating the elimination of some 5—10% of non-uronide contaminants, the precipitation processes had not reduced the nitrogen content, and the ash contents were not significantly reduced. Other purification methods were therefore investigated. It is well-known that bi- and ter-valent ions can cause gel-formation and cross-linking; [5] ash-free samples are therefore required for some analyses (cf. ref. 12).

Purification by Electrodialysis.—A portion of each crushed nodule was electrodialysed [7] (as 2% aqueous solution), ion-exchange membranes being used.[14] Cooling coils in the electrodialysis compartments prevented the temperature of the gum from rising above 30°. Trial experiments (on sample IX) showed that electrodialysis for 6 hr. (cf. ref. 7) was required to achieve the low ash values shown in Table 3. Since ash determinations at the 0·01% level consume relatively large amounts of material, determinations were not made on all samples.

After electrodialysis for several hours, the gum solutions separated into a clear, colourless upper layer and a viscous, slightly coloured lower layer. The upper layer was removed by

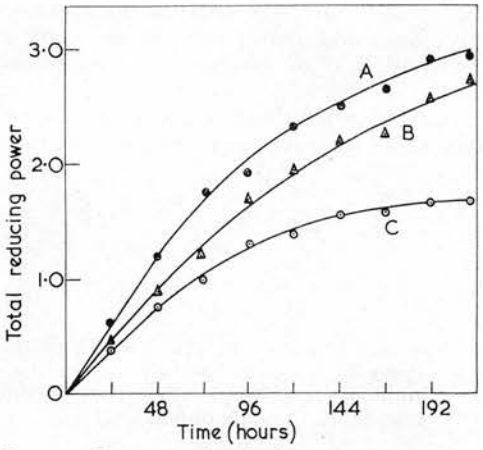

FIG. 1. Increase of reducing-power on autohydrolysis of (A) nodule (VII), (B) nodule III, and (C) nodule V. Reducing power is expressed as mg. of arabinose per 2 ml. of 1% solutions.

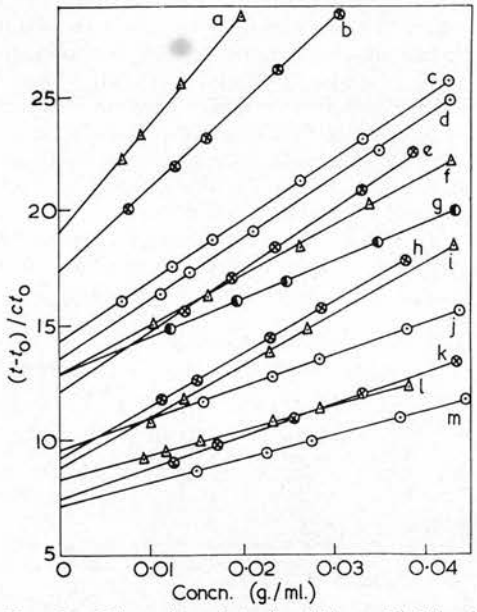

FIG. 2. Viscosity plots (in 4% aq. NaCl) of crude and purified samples. (a) VIII, crude. (b) VIII, pptd. (c) VIII, electrodialysed. (d) III, electrodialysed. (e) III, pptd. (f) III, crude. (g) III, ion-exchange. (h) II, pptd. (i) III, crude. (j) II, electrodialysed. (k) V, pptd. (l) V, crude. (m) V, electrodialysed.

pipette and found to contain only traces of gum. This effect may be worthy of further examination, since disintegration of a complex coacervate may be involved (cf. ref. 5).

Studies on Electrodialysed Samples.—The results obtained are compared in Table 3. Although the ash content had been effectively reduced, no elimination of nitrogen was achieved.

TABLE 3.

Determinations on electrodialysed samples.

	I	II	III	IV	V	VI	VII	VIII	IX
Ash (%)	0·02	0·01	n.d.	n.d.	0·02	0·02	0·03	n.d.	0·05
	0·02	0·02			0·03	0·03	0·04		0·05
Nitrogen (%)	0·15	0·11	0·16	0·17	0·10	0·11	0·19	0·17	0·17
	0·16	0·11	0·16	0·17	0·10	0·10	0·19	0·18	0·17
Uronic anhydride (%)	13·6	13·0	13·5	13·8	12·1	12·5	16·8	13·4	13·8
	13·7	13·3	13·6	13·6	12·2	12·3	16·6	13·5	13·8
Limiting flow-time no.	12·0	9·5	13·5	n.d.	7·0	12·4	13·6	14·2	n.d.

Potentiometric titrations showed that the ash-free gum behaved as a strong acid [7] (pH of a 1% aqueous solution = 2·9), and the values obtained for the neutralisation equivalent indicated that all the acidity arose from the uronic acid groups (*e.g.*, Found, for sample VIII: Neut.

equiv., 1340; uronic anhydride = 13·5%. Required; Neut. equiv., 1300 if all acidity is due to uronic acid groups).

Purification by Ion-exchange.—A dilute aqueous solution of nodule III was filtered, then de-ionised [15] by passage through a column of the cation-exchange resin " ZeoKarb 225." Analysis of the freeze-dried eluate gave: ash 2·4%, nitrogen 0·14%, uronic anhydride 13·5%, $[\alpha]_D$ +59°. Viscosity determinations gave the plot shown in Fig. 2g. This ion-exchange method was not applied to the other samples since the purification achieved did not approach that given by electrodialysis.

Comparison of the Viscosity Behaviour of Samples before and after Purification.—Samples were examined carefully to assess the extent of inter-nodule variation and the effect on each nodule of the various purification procedures. The viscosity plots for the crude and the purified samples of nodules II, III, V, and VIII are shown in Fig. 2; these curves are typical and represent the range of behaviour observed.

Fractionation Experiments on Aqueous Solutions of the Gum.—(1) *Chemical precipitation methods.* No useful fractionation resulted from (*a*) graded addition of ethanol, (*b*) addition of iodine–potassium iodide reagent (cf. ref. 16), or (*c*) addition of cetyltrimethylammonium bromide [17] at pH 4, 7, or 9.

(2) *Electrophoresis.* Several experiments were made with glass-fibre paper in 2M-sodium hydroxide at 1000 v for 6—18 hr. Movements of several cm. resulted, but there was no distinct separation of components (cf. ref. 3).

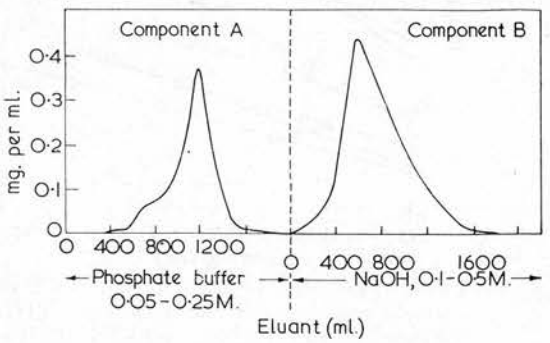

FIG. 3. Elution pattern from fractionation of a single nodule on diethylaminoethylcellulose. The broken line denotes change of solvent.

(3) *Chromatography on diethylaminoethylcellulose.*[18] A solution of sample I (electrodialysed, 360 mg.) was treated on a column (40 × 5 mm.) of diethylaminoethylcellulose; gradient elution with phosphate buffer (pH 4·6, 0·05M ⟶ 0·25M) was used, followed by gradient elution with aqueous sodium hydroxide (0·1M ⟶ 0·5M). The average flow-rate was ~40 ml. per hr. Fractions (40 ml.) were screened by the phenol method.[19] Fig. 3 shows the elution pattern observed. The total recovery from the column was 331 mg.: component A (117 mg., 35%) and component B (214 mg., 65%) contained 12·5% and 15·4% of uronic anhydride, respectively.

Sample II (electrodialysed, 220 mg.) gave an elution pattern similar to that shown in Fig. 3. Component A (66 mg., 34%) and component B (126 mg., 66%) had uronic anhydride contents of 12·4% and 14·9%, respectively.

Sample III (electrodialysed, 460 mg.) similarly gave 180 mg. (41%) of component A (uronic anhydride, 12·6%) and 258 mg. (59%) of component B (uronic anhydride, 15·2%).

Hydrolysis: Percentages of Sugars Present.—Treatment with 2N-sulphuric acid at 90—95° for 8 hr. completely hydrolysed the gum. Sample IX gave galactose 38%, arabinose 46%, rhamnose 3%, and glucuronic acid 12·5% (expressed as approximate percentages; cf. ref. 20). The method of determining the sugar ratios involves the separate stages of hydrolysis, neutralisation, reduction in volume, chromatographic separation, elution, and estimation of reducing power: it is considered that the results cannot be more accurate than ±5—10% of the actual percentage present.

For samples I—VIII, however, the results, particularly for the rhamnose content, varied by amounts which are considered to be outside the possible experimental error. The two most widely differing nodules were samples V and VII. Sample V gave glucuronic acid 11%,

galactose 42%, arabinose 47% and rhamnose 1%: sample VII gave glucuronic acid 16%, galactose 34%, arabinose 42%, and rhamnose 8%.

Discussion

The gum from *A. seyal* is similar to the *Acacia* gums previously studied in containing glucuronic acid, galactose, arabinose, and rhamnose. The presence of acid-labile residues and the marked decrease in viscosity detected on mild hydrolysis indicate that *A. seyal* probably further resembles other *Acacia* gums in having a main chain, resistant to hydrolysis, to which is attached acid-labile side-chains. Of the *Acacia* gums studied to date, all have given negative optical rotations with the exception of *A. karroo*,[20] to which must now be added *A. seyal*. The methoxyl content of the *A. seyal* nodules examined varied from 0·5% to 1·5%; only *A. mollissima* [21] has previously been reported to have a methoxyl content (0·35%). A methoxyl content of 1% has been found [22] to be significant in *Khaya grandifolia* gum.

The results presented in Tables 1—3 indicate that the inter-nodule variation in composition is greater than can be explained on the basis of possible analytical error. The variation is similar in extent to that previously found [11] for nodules of C. *leonense* gum.

The nodules examined were collected and authenticated by an expert on the identification of *Acacia* species. It may otherwise have been suggested that nodules V and VI (from their appearance), nodule VII (uronic acid content), and nodule VIII (viscosity) originated from some species other than *A. seyal*. However, the data for each nodule, taken as a whole, leave little basis for doubting the authenticity of the samples. Taken jointly, the nitrogen content and the optical rotation of an *Acacia* gum are strongly indicative of its species: preliminary studies of other Sudanese *Acacia* species such as *A. arabica, A. laeta, A. dealbata, A. drepanolobium*, and *A. campylacantha* (which have not been studied previously) have shown that the nitrogen content of *A. seyal* is characteristically low, and, moreover, is not reduced by any of the methods of purification used. The mechanism of gum formation is still far from clear,[6] and further knowledge of the nature of the nitrogen content in plant gums would be of value in assessing the relative importance of the enzymic polymerisation theory [5] in relation to the alternative theories [6,23] that gum formation results from (*a*) normal plant metabolism or (*b*) pathological reactions to resist invading micro-organisms or to avoid loss of moisture.[5]

Although it has been reported that the ash content of some species of gum can be eliminated [10,22] by precipitation methods, our experiments with *Acacia* species have shown that their ash content cannot be reduced by more than about 50%, even after 4 re-precipitations. The results reported for *A. seyal* are typical in this respect. Electrodialysis is the most effective method of reducing the ash content to a very low value; as shown in Fig. 2, the most viscous nodule (VIII) showed a marked decrease in viscosity on purification, although the other nodules were not affected to a comparable extent. In general, the purification methods studied do not appear to alter significantly the physical properties of the gum.

Fractionation of *A. seyal* gum on diethylaminoethylcellulose gave two components having uronic anhydride contents of 12·5% and 15·3%, respectively; the close similarity of the elution patterns suggested that different nodules contained the same two components in slightly varying proportions. Conclusive evidence of heterogeneity is often difficult to achieve. Indeed, conflicting results may be given by different techniques; trypsin is electrophoretically heterogeneous, although only one component was evident on examination by the ultracentrifuge.[24] For gum arabic,[1] chemical fractionation has been less successful than immunochemical experiments. Our failure to separate the components of *A. seyal* by electrophoresis (cf. ref. 3) may therefore be explained by the fact that, in single nodules, the two components do not differ sufficiently in uronic acid content, upon which electrophoretic movement must depend to a large extent.[25] Studies of the chemical structure of the two components of this gum are in progress.

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EDINBURGH, 9. [*Received, March 9th*, 1962.]

[1] Heidelberger, Adams, and Dische, *J. Amer. Chem. Soc.*, 1956, **78**, 2853.
[2] Joubert, *J. S. African Chem. Inst.*, 1954, **7**, 107.
[3] Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.
[4] Hirst, 4th Internat. Congress of Biochemistry, Vienna, 1958.
[5] Whistler, " Industrial Gums," Academic Press, New York, 1959.
[6] Smith and Montgomery, " The Chemistry of Plant Gums and Mucilages," Reinhold Publ., Inc., New York, 1959.
[7] *E.g.*, Thomas and Murray, *J. Phys. Chem.*, 1928, **32**, 676.
[8] Butler and Cretcher, *J. Amer. Chem. Soc.*, 1931, **53**, 4160.
[9] Anderson and Sands, *Adv. Carbohydrate Chem.*, 1945, **1**, 329.
[10] Hirst and Jones, *J.*, 1938, 1174.
[11] Anderson, Hirst, and King, *Talanta*, 1959, **3**, 118.
[12] Greenwood and Mathieson, *Chem. and Ind.*, 1956, 191.
[13] Anderson and Duncan, *Talanta*, 1961, **8**, 241.
[14] Anderson and Wylam, *Chem. and Ind.*, 1956, 191.
[15] Hamilton, Spriesterbach, and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 443.
[16] Whistler and Gaillard, *Arch. Biochem. Biophys.*, 1961, **93**, 332.
[17] Scott, *Chem. and Ind.*, 1955, 168.
[18] Neukom, Heri, Kundig, and Deuel, *Helv. Chim. Acta*, 1960, **43**, 64.
[19] Dubois, Gilles, Hamilton, Rebers, and Smith, *Analyt. Chem.*, 1956, **28**, 350.
[20] Charlson, Nunn, and Stephen, *J.*, 1955, 1428.
[21] Stephen, *J.*, 1951, 646.
[22] Aspinall, Hirst, and Mathieson, *J.*, 1956, 989.
[23] Jones and Smith, *Adv. Carbohydrate Chem.*, 1949, **4**, 243.
[24] Perrone, Disitzer, and Dormont, *Nature*, 1959, **183**, 605.
[25] Cf. Colvin, Cook, and Adams, *Canad. J. Chem.*, 1952, **30**, 603.

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BUNGAY, SUFFOLK.

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Applications of infrared spectroscopy—VIII*: Investigation of a reported anomalous Zeisel alkoxyl reaction

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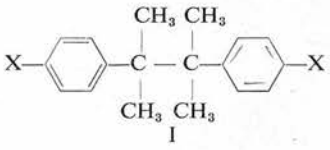
SEVERAL examples of molecules which react anomalously in the Zeisel alkoxyl reaction have been reported,[1,2,3] and solvent retention is known[4,5,6] to be a potential source of error in alkoxyl determinations.

Huang and Morsingh[7] reported that 2,3-dimethyl-2,3-diphenylbutane (I, X = H) and certain of



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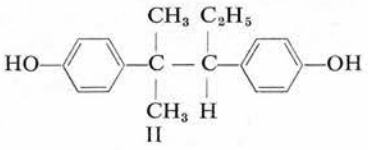

II

its derivatives (I, X = OH, NO_2) reacted anomalously in the Zeisel reaction, giving an apparent methoxyl content of 3·3%. Such a result appeared surprising; when it was observed that the experimental results quoted[7] were somewhat variable, and that the specimen used[7] had been prepared by a method[8] involving crystallisation from ethanol, it appeared that the reported anomaly might be, in effect, a further example of solvent retention. If so, application of the infrared alkoxyl method[9] would reveal that ethyl iodide, and not methyl iodide, was the reaction product.

2,3-Dimethyl-2,3-diphenylbutane, prepared by Farmer and Moore's method,[8] was re-crystallised from ethanol. After drying in the normal way, the product had m.p. 118° (lit., 118–119°). Analysis (Weiler and Strauss, Oxford,) England gave %C = 90·51 %H =, 9·34; required, %C = 90·75, %H = 9·25. When treated with constant-boilinghydrio dic acid, under the conditions described by Anderson and Duncan,[9] this compound gave no volati le reaction products, even after prolonged reflux overnight. Indeed, so stable is this hydrocarbon that it was recovered unchanged (identity of infrared spectrum) from the hydriodic acid reaction medium. This compound therefore neither retains solvent of crystallisation nor reacts anomalously under *normal* Zeisel conditions.

It is perhaps significant that Huang and Morsingh reported[7] that neither the dimethoxy derivative (I, X = OCH_3) nor compound II reacted anomalously, and that drastic conditions, normally reserved for the analysis of *N*-methyl groups, were used[10] in their analyses. Thus the sample was dissolved in phenol and acetic anhydride and refluxed with hydriodic acid; after evaporation to dryness, the residue was heated above 300°. It is quite unreasonable for results obtained by such a procedure to be described[10] as anomalous Zeisel methoxyl determinations.

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Summary—2:3-dimethyl-2:3-diphenylbutane is stable to reflux with constant-boiling hydriodic acid under standard Zeisel alkoxyl reaction conditions. A previous report that this compound reacts anomalously is therefore incorrect.

Zusammenfassung—2,3-Dimethyl-2,3-diphenylbutan ist stabil, wenn es unter Rückfluss mit konstantsiedender Salzsäure gemäss den Bedingungen einer Alkoxylbestimmuhg nach Zeisel gekocht wird. Eine frühere Mitteilung, dass die Verbindung abnormales Verhalten zeigt, ist daher unrichtig.

Résumé—Le 2-3-diméthyl-2-3-diphénylbutane est stable quand il est chauffé au reflux avëc de l'acide iodhydrique bouillant constamment dans les conditions de la réaction standard de Zeisel pour les alcoyles. Un rapport antérieur prévoyant que ce composé réagit de facon anormale est donc incorrect.

* Part VII: *Talanta*, 1961, **9**, 611.

REFERENCES

[1] H. Gysel, *Mikrochim. Acta*, 1954, 743.
[2] D. M. W. Anderson and J. L. Duncan, *Chem. and Ind.*, 1959, 457.
[3] N. Karpitschka, *Mikrochim. Acta*, 1961, 738.
[4] D. M. W. Anderson and J. L. Duncan, *Talanta*, 1961, **8**, 241.
[5] D. M. W. Anderson and N. J. King, *ibid.* 1961,, **8**, 497.
[6] D. W. Drummond and E. E. Percival, *J. Chem. Soc.*, 1961, 3908.
[7] R. L. Huang and F. Morsingh, *Analyt. Chem.*, 1952, **24**, 1359.
[8] E. H. Farmer and C. G. Moore, *J. Chem. Soc.*, 1951, 141.
[9] D. M. W. Anderson and J. L. Duncan, *Talanta*, 1960, **7**, 70.
[10] R. L. Huang and L. Kum Tatt, *Analyt. Chem.*, 1955, **27**, 1030.